AFAMRL-TR-80-102




# ENVIRONMENTAL QUALITY RESEARCH FISH AND AUFWUCHS BIOASSAY

## Fifth Annual Report

*STEPHEN A. KLEIN*
*DAVID JENKINS*
*THE REGENTS OF THE UNIVERSITY OF CALIFORNIA*
*UNIVERSITY OF CALIFORNIA, IRVINE*
*IRVINE, ORANGE COUNTY, CALIFORNIA 92664*

JANUARY 1981



Approved for public release; distribution unlimited.

AIR FORCE AEROSPACE MEDICAL RESEARCH LABORATORY
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433

## NOTICES

When US Government drawings, specifications, or other data are used for any purpose other than a definitely related Government procurement operation, the Government thereby incurs no responsibility nor any obligation whatsoever, and the fact that the Government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Please do not request copies of this report from Air Force Aerospace Medical Research Laboratory. Additional copies may be purchased from:

National Technical Information Service
5285 Port Royal Road
Springfield, Virginia 22161

Federal Government agencies and their contractors registered with Defense Documentation Center should direct requests for copies of this report to:

Defense Documentation Center
Cameron Station
Alexandria, Virginia 22314

## TECHNICAL REVIEW AND APPROVAL

AFAMRL-TR-80-102

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals, "Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

**FOR THE COMMANDER**

ANTHONY A. THOMAS, MD
Director
Toxic Hazards Division
Air Force Aerospace Medical Research Laboratory

AIR FORCE/56780/26 February 1981 — 150

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

| REPORT DOCUMENTATION PAGE | | READ INSTRUCTIONS BEFORE COMPLETING FORM |
|---|---|---|
| 1. REPORT NUMBER<br>AFAMRL-TR-80-102 | 2. GOVT ACCESSION NO. | 3. RECIPIENT'S CATALOG NUMBER |
| 4. TITLE *(and Subtitle)*<br>Environmental Quality Research<br>Fish and Aufwuchs Bioassay<br>Fourth Annual Report | | 5. TYPE OF REPORT & PERIOD COVERED<br>Annual Report<br>1 June 1979 – 31 May 1980<br>6. PERFORMING ORG. REPORT NUMBER |
| 7. AUTHOR(s)<br>Stephen A. Klein<br>David Jenkins | | 8. CONTRACT OR GRANT NUMBER(s)<br>F 33615-76-C 5005 |
| 9. PERFORMING ORGANIZATION NAME AND ADDRESS<br>The Regents of the University of California<br>University of California, Irvine<br>Irvine, Orange County, California 92664 | | 10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS<br>62202F, 6302, 04, 17 |
| 11. CONTROLLING OFFICE NAME AND ADDRESS Air Force<br>Aerospace Medical Research Laboratory,<br>Aerospace Medical Division, Air Force Systems<br>Command, Wright-Patterson AFB, Ohio 45433 | | 12. REPORT DATE<br>January 1981<br>13. NUMBER OF PAGES<br>96 |
| 14. MONITORING AGENCY NAME & ADDRESS *(if different from Controlling Office)* | | 15. SECURITY CLASS. *(of this report)*<br>UNCLASSIFIED<br>15a. DECLASSIFICATION/DOWNGRADING SCHEDULE<br>N/A |

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

Fish
Bioassay
Toxicity
Aufwuchs
JP-4
JP-8
Shale JP-8

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

This report contains the results of research efforts of a project concerned with defining the effects of potential environmental contamination resulting from the use of certain Air Force materials on fresh water and saline water fish and attached periphyton (aufwuchs). Materials being evaluated include JP-4, JP-8, and shale JP-8. Techniques for exposing organisms to these substances are discussed and results of such exposures are presented.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

## SUMMARY AND CONCLUSIONS

This report is concerned with the toxic effects of three hydrocarbon fuels (JP-4, JP-8, and shale JP-8) on saline water organisms, and with the toxicity of shale JP-8 to fresh water fish. Specifically, the following topics are covered:

The toxicity of the shale oil derived fuel, shale JP-8, to the fresh water species rainbow trout (Salmo gairdneri) and to flagfish (Jordanella floridae).

A discussion of the relative toxicity of the three hydrocarbon fuels to fresh water fish.

The toxicity of each fuel to the estuarine species stickleback (Gasterosteus aculeatus) and aufwuchs.

A discussion of the relative toxicity of the three fuels to estuarine species.

Results of a continuous-flow, partial chronic bioassay of 119 days duration of the water soluble fraction (WSF) of shale JP-8 to rainbow trout supported the following conclusions:

There was no significant effect on the success of egg hatching in the range of concentrations examined (0—1.29 ± 0.36 mg WSF of shale JP-8/ℓ).

There was an acceleration in egg hatching rate for WSF of shale JP-8 concentrations ≥ 0.60 mg/ℓ.

The no-effect level on rainbow trout growth was < 0.13 ± 0.06 mg WSF of shale JP-8/ℓ (the lowest level tested).

The no-effect level on rainbow trout survival was between 0.13 and 0.32 mg WSF of shale JP-8/ℓ.

WSF of shale JP-8 accumulation in rainbow trout whole body tissue was low (mean accumulation ratio = 72) compared with other hydrocarbon fuels (JP-4, JP-8, and JP-9) that have been examined.

There was no indication of WSF of shale JP-8 accumulation in rainbow trout livers, a result contrary to the preferential accumulation of other hydrocarbon fuels in liver rather than muscle tissue.

Results of a continuous-flow partial chronic bioassay of 148 days duration of the WSF of shale JP-8 to flagfish supported the following conclusions:

There was no effect on the success of egg hatching in the range of WSF of shale JP-8 concentrations examined (0—1.35 ± 0.35 mg/ℓ).

The rate of egg hatching was accelerated at WSF of shale JP-8 concentrations greater than 0.08 ± 0.04 mg/ℓ.

There was no effect of fish growth on survival at a WSF of shale JP-8 concentration of 0.51 ± 0.30 mg/ℓ.

Significant effects on fish growth and survival were observed at a WSF of shale JP-8 concentration of 1.35 ± 0.35 mg/ℓ.

There was no observed effect on reproductive success at WSF of shale JP-8 concentrations of 0.51 ± 0.30 mg/ℓ.

Results of continuous-flow bioassays of the WSF of hydrocarbon fuels to stickleback supported the following conclusions:

At WSF of JP-4 concentrations of ≥ 8.9 mg/ℓ 100% mortality occurred in a 7-day study. The 168-hr LC 50 was 6.80 mg/ℓ with 95% confidence limits of 6.14 to 7.53 mg/ℓ.

At WSF of shale JP-8 concentrations of 2.5 mg/ℓ 100% mortality occurred in a 14-day study. The 336-hr LC 50 was 1.45 mg/ℓ with 95% confidence limits of 1.24 to 1.70 mg/ℓ. The 168-hr LC 50 was estimated to be 1.95 mg/ℓ.

WSF of JP-8 concentrations in the range of 0 to 3.8 mg/ℓ did not cause 100% mortality. The 336-hr LC 50 was 3.22 mg WSF of JP-8/L with 95% confidence limits of 2.75 to 3.76 mg/ℓ. The no-effect level was <0.8 mg WSF of JP-8/ℓ (the lowest level tested).

Results of continuous-flow bioassays of the WSF of hydrocarbon fuels to aufwuchs supported the following conclusions:

In a 21-day study the no-effect level of WSF of JP-4 to aufwuchs growth and productivity was < 3.0 mg/ℓ (the lowest level tested).

In a 14-day study the no-effect level of WSF of shale JP-8 on aufwuchs growth was between 0 and 0.8 mg/ℓ. Aufwuchs productivity was affected at WSF of shale JP-8 concentrations between 0.8 and 1.5 mg/ℓ. Toxic effects at 1.5 mg WSF of shale JP-8/ℓ were far more severe to aufwuchs than were the toxic effects of 3.0 mg WSF of JP-4/ℓ.

In a 14-day study the no-effect level of WSF of JP-8 on aufwuchs productivity was 0.7 to 1.4 mg/ℓ. The no-effect level on aufwuchs biomass was in the range of 2.7 to 3.3 mg WSF of JP-8/ℓ.

# PREFACE

The research reported herein was conducted at the Sanitary Engineering Research Laboratory, University of California at Berkeley, under the terms of contract F 33615-76-C5005 with the U. S. Air Force. The contract monitor was Lt. Col. C. B. Harrah, Air Force Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio. Professors David Jenkins and Robert C. Cooper were the Principal Investigators. Mr. Stephen A. Klein was the project manager. Ms. P. C. Ulrichs and Mr. Robert Okazaki were responsible for the conduct of the bioassays.

Mr. Tao-Kuan Tien, Mr. Jon Palm, Mr. Francis Jenq, and Ms. Lynn De Lain, graduate students, served as research assistants.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

| Table | LIST OF TABLES | Page |
|---|---|---|

# INTRODUCTION

Studies included in this report are directed toward providing information on the toxicity to aquatic life of the kerosene-based jet fuels, JP-4 and JP-8, and a fuel derived from shale oil, designated as shale JP-8. JP-4 and JP-8 are currently in use, and shale JP-8 is under consideration for use by the U. S. Air Force.

To define the environmental impact of a toxicant on aquatic life, experimental protocols have been established for fresh water and saline water organisms. The fresh water protocol includes conducting acute static bioassays of 96-hr duration, acute continuous-flow bioassays of 2-weeks duration, and chronic continuous-flow bioassays of 4- to 6-months duration on warm-water and cold-water fish. The toxicity of JP-4 and JP-8 to fresh water fish has been reported in previous work (February 1975 First Annual Report AMRL-TR-74-82, October 1976 Annual Report AMRL-TR-76-64, November 1977 Second Annual Report AMRL-TR-77-54, November 1978 Third Annual Report AMRL-TR-78-65 and November 1979 Fourth Annual Report AMRL-TR-79-70). In this report the toxicity of shale JP-8 to fresh water fish is examined. Included are preliminary range-finding studies of the toxicity of the water soluble fraction (WSF) of shale JP-8 to the warm water fish golden shiner (Notemigonus chrysoleucas) and a partial chronic bioassay of 4 months duration on the effect of the WSF of shale JP-8 to the cold water species, rainbow trout (Salmo gairdneri). This study commenced with "eyed" eggs and examined the hatching, the growth, and development of fry and concluded with analysis of fish tissue for fuel accumulation. A chronic study of 5 months duration on the toxicity of the WSF of shale JP-8 to warm water flagfish is also presented, and examines egg hatching, fry growth, and development and reproductive ability.

Studies on JP-4, JP-8, and shale JP-8 have been conducted in saline water from San Francisco Bay at the large-scale bioassay facility ("analog" facility) of the Sanitary Engineering Research Laboratory. The experimental protocol includes performing acute static bioassays of 96-hr duration and 14-day continuous-flow bioassays on the toxic effects of the WSF of each fuel to the 3-spine stickleback (Gasterosteus aculeatus) and aufwuchs (attached periphyton growth). Fuel volume constraints dictated the use of small-scale bioassay vessels (40-$\ell$ working capacity) for these studies instead of the original facility of 4.2-$m^3$ capacity analog tanks which had been suitable for the previously reported rocket fuel (hydrazines) toxicity work (November 1978 Third Annual Report AMRL-TR-78-65). Due to complications (detailed in the body of this report) the small-scale vessels did not permit the simultaneous study of stickleback and aufwuchs, which was the customary procedure followed with the hydrazines. Segregated studies are therefore presented of each species exposed to each of the three fuels.

The discussion section of this report compares the toxicity of all hydrocarbon fuels studied to date on both fresh water and saline water species. Included is a discussion of the relative toxicity of JP-4, JP-8, shale JP-8, and JP-9 and its components methylcyclohexane (MCH), RJ-4 and RJ-5 to flagfish and to rainbow trout. The relative toxicity of JP-4, JP-8, and shale JP-8 to stickleback and aufwuchs is also discussed.

# MATERIALS AND METHODS

## INTRODUCTION

The majority of the materials and methods employed in this work have been detailed in previous annual reports (November 1977 Second Annual Report AMRL-TR-77-54, November 1978 Third Annual Report AMRL-TR-78-65, and November 1979 Fourth Annual Report), and will not be repeated here. New materials, apparatus, and techniques discussed here are shale oil derived JP-8 (shale JP-8) fuel, the quantitative analytical method for shale JP-8 and its precision and the apparatus required to expose saline water organisms to the water soluble fraction (WSF) of hydrocarbon fuels.

## SHALE JP-8

This material, which was derived from shale oil, was supplied by the U.S. Air Force. The fuel was designated "shale JP-8" because of the similarity of its major components to those present in JP-8.

## QUANTITATIVE ANALYSIS OF WSF OF SHALE JP-8

### Method Selection

The purge-and-trap and pentane extraction methods presently used to quantify WSF of JP-4 and WSF of JP-8, respectively, were investigated to evaluate their applicability to the quantitative analysis of WSF of shale JP-8.

Equivalent volumes of a sample of WSF were analyzed by the two methods. By pentane extraction (two extractions of a 300-mℓ sample with 25-mℓ volumes of pentane) a result of 4.53 mg/ℓ (100% of total) was obtained; by the purge-and-trap method a value of 0.345 mg/ℓ resulted. Thus, the purge-and-trap method recovered only 7.6% of the quantity attained by the pentane extraction method.

A chromatogram of a pentane extracted sample (Figure 1) indicates that the predominant compounds comprising shale JP-8 are above $C_9$. This explains the poor purging efficiency of shale JP-8 because only compounds in the range of $C_6-C_9$ normal alkanes are 100% purged, and above $C_9$ the purging efficiency begins to decline. In contrast, JP-4 predominates in compounds in the $C_6-C_9$ range and can be recovered with 98% efficiency by the purge method.

The relatively low volatility of shale JP-8 resulted in small losses during the rotary evaporation of pentane extract in the pentane extraction method. Tests showed that there was no loss at all in reducing 70 mℓ pentane to as little as 0.2 mℓ. This step caused a loss of 55% JP-4 and 33% JP-8 when reductions were made to 3-mℓ volume.

Number of Pentane Extractions Required. The extraction efficiency of pentane was examined by extracting a sample five times (Sample A). Another aliquot of the sample (Sample B) was extracted once and then the aliquot was extracted four more times (Sample C). Samples A, B, and C were each

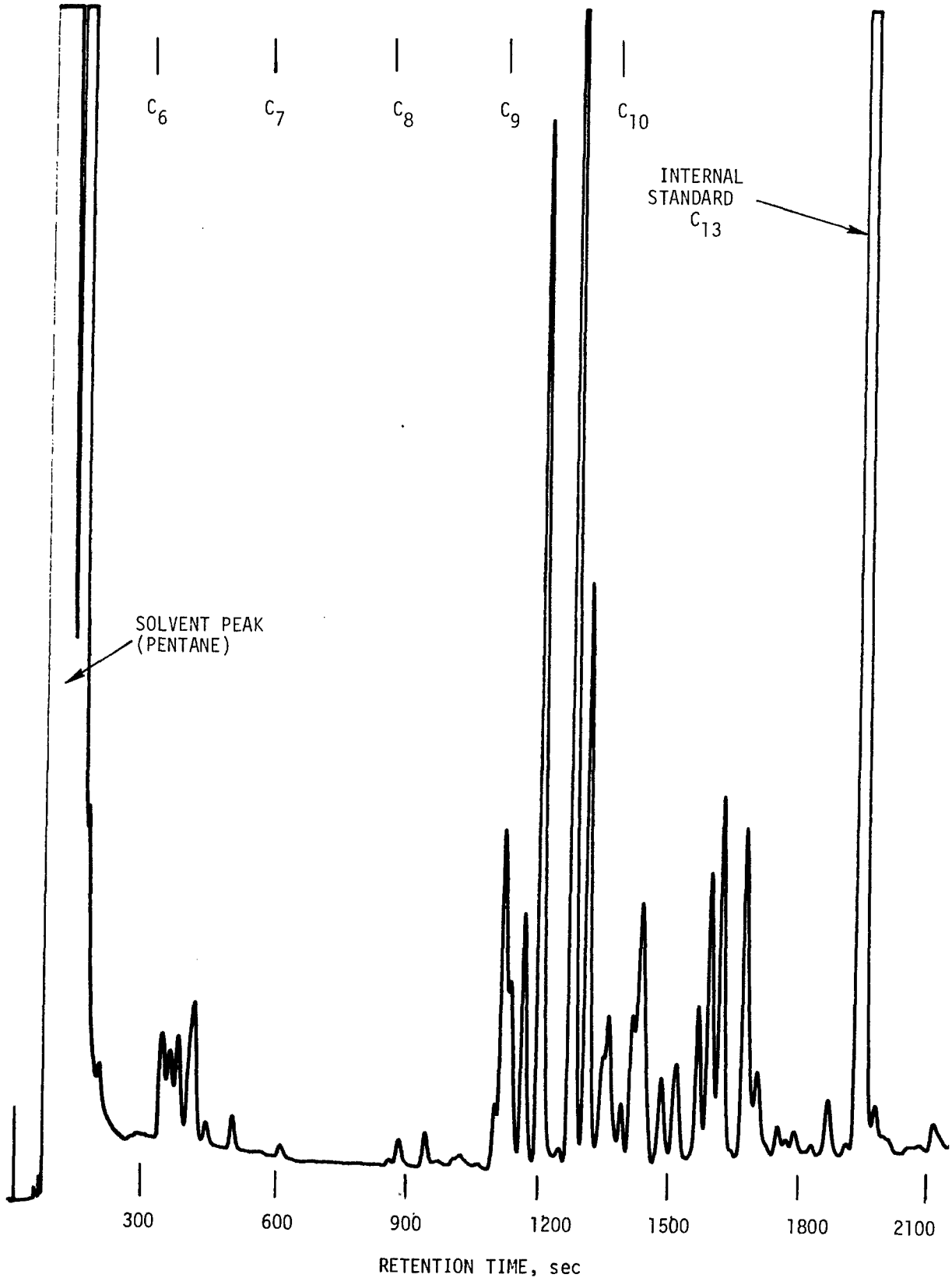



FIGURE 1. CHROMATOGRAM OF WSF OF SHALE JP-8, TEMPERATURE PROGRAM: 2 MIN INITIAL DELAY, 60°C; 4°C/MIN INCREASE TO 200°C

rotary evaporated to 3 mℓ and then analyzed by gas chromatography. The results were as follows:

| Sample Number | mg/ℓ |
|---|---|
| A | 1.21 |
| B | 1.27 |
| C | 0.006 |

The single extract, Sample B, was equivalent to the quintuple extract, Sample A, indicating that a single extraction is sufficient. This was further confirmed by the results for Sample C which showed that virtually no more shale JP-8 could be recovered by four more extractions of Sample B.

## Method Reproducibility

The reproducibility of the pentane extraction method was examined by taking ten 300-mℓ aliquots of a dilute sample and applying the single pentane extraction to each, followed by reduction to 0.5 mℓ and analysis by gas chromatography. A mean concentration ($\bar{x}$) of 0.484 mg/ℓ, with a standard deviation (s) of 0.029 mg/ℓ and a percent relative standard deviation (RSD) of 5.99 was obtained.

## Injection Reproducibility

Ten injections of one of the previous samples were made with the following results: $\bar{x}$ = 0.4295 mg/ℓ; s = 0.0077 mg/ℓ; and RSD = 1.78%.

## Reduction Volume of Pentane Extract

The normal volume reduction to 3 mℓ was found to be insufficient to meet GC detector sensitivity requirements – particularly for low concentration samples. The fact that the maximum WSF concentration of shale JP-8 was approximately 4.5 mg/ℓ (compared with 12 mg/ℓ for normal JP-8) meant that serial dilutions of the WSF produced a much lower range of concentrations than previously. For shale JP-8, tests indicated that the pentane extract could be reduced to as little as 0.1 mℓ without volatility losses and with improved detection sensitivity. In samples below 10% of the maximum WSF concentration, the initial sample volume had to be increased from 300 to 600 mℓ.

## Internal Standard

The internal standard selected for sample analysis was the $C_{13}$ n-alkane, tridecane. Although shale JP-8 contains some compounds that chromatograph in this region, the WSF contains only a very small amount of such compounds. This minor disadvantage was outweighed by the advantages of selecting a lower molecular weight compound in terms of time saved in chromatographic analysis and improved analytical accuracy. To override the presence of small peaks in the region of the standard peak, the concentration of standard introduced to each sample was increased five-fold

over that previously used. Standard peaks were thus on the order of 500,000 mv· sec which reduced the error to insignificance.

## BAY WATER SOURCE AND APPARATUS

### Source

Central San Francisco Bay water was delivered to bioassay facilities from an intake 1.04 km offshore.

### Solubilizers

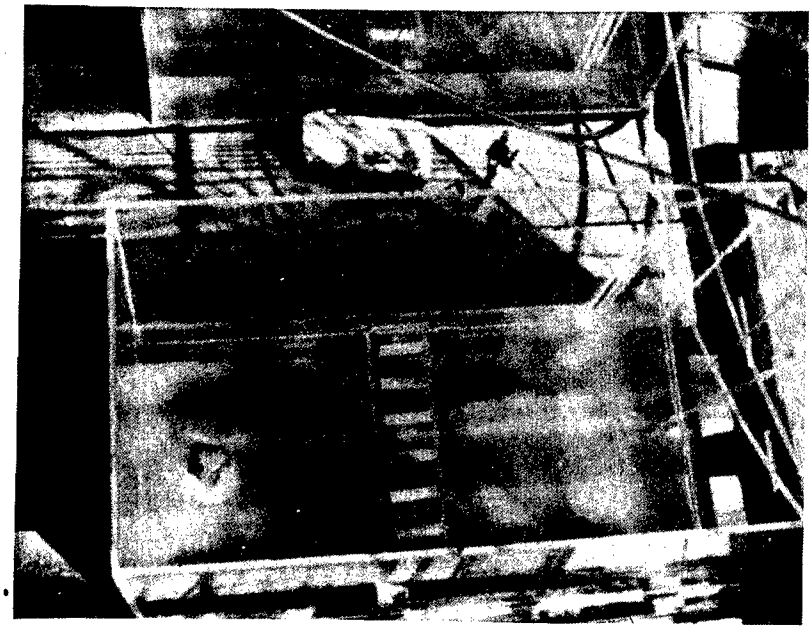
Two fuel solubilizers were constructed to produce a continuous flow of Bay water saturated with WSF of hydrocarbon fuels. The solubilizers are nearly identical to those currently in use in fresh water studies (Krugel et al., 1977) and each consisted of 5 glass columns (each 1.2 m long and 3.8 cm in diameter) connected in series. A 3.05-m high platform supported by unistrut beams was constructed for accommodating a head tank, solubilizers, and fuel filters. The upper platform was a sheet of 1.22 m by 2.44 m by 2.54 cm plywood coated with epoxy paint. The head tank was on a 0.61-m high table anchored to the platform, and provided gravity feed for the entire system. The apparatus is shown in Figure 2A.

### Filtration-Sterilization System

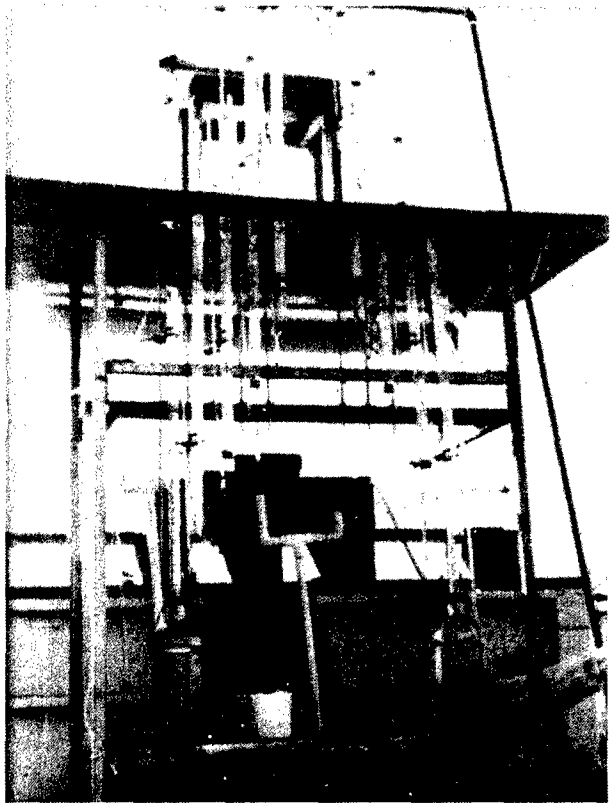
To prevent clogging of the solubilization and distribution system by particulate matter and algal growths, the seawater was passed through a settling tank, a sand filter, and an ultra-violet sterilizer unit prior to delivery into the head tank. The settling tank, with dimensions of 1.2 m by 1.2 m by 44 cm deep, was constructed of 1.9-cm plywood coated with epoxy paint. The tank was baffled to aid in the removal of silt and debris. Seawater flowed from the settling tank into a Model 1 FM 24 Jacuzzi sand filter (Jacuzzi Bros., Inc., San Leandro, CA) equipped with a 1 hp pump, a 0.61-cm diameter tank, and 929 $cm^2$ of filter area. The pump can deliver a flow rate of 60 gpm through the filter and backwash at a flow rate of 46 gpm. A float switch to control the filter pump has been installed in the settling tank to prevent seal damage of the pump in the event of interruption of seawater flow. The filtration system is a dual media arrangement consisting of 21.6 cm of U.S. No. 40 sand and 7.6 cm of Anthrafilt No. 1 supported on a 16.5-cm layer of 0.3–0.6-cm gravel.

After filtration the seawater is sterilized by a Model L-150 UV sterilizer (Ultraviolet Technology, Inc. of Encinitas, CA) with a maximum flow capacity of 67 gpm and an estimated dosage of 12,000 $\mu$-watts/$cm^2$. Two wooden housing units coated with epoxy paint protect the filter pump and the sterilizer unit from the weather.

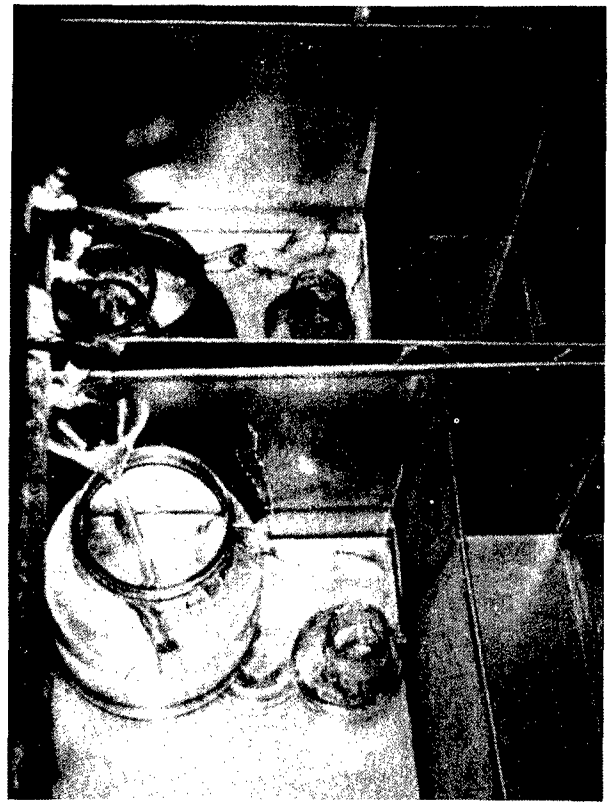
A series of tests was performed to evaluate the efficiency of the seawater sand filter and ultraviolet units. A known concentration of the bacterium Escherichia coli B and a poliovirus was mixed with clean fresh seawater. After sampling the units were operated until approximately 312 $\ell$ of the "spiked"



2B. BAY WATER EXPOSURE TANK (WITH AUFWUCHS RACK)



2A. BAY WATER FUEL SOLUBILIZERS



2C. FRESH WATER CIRCULATION SYSTEM FOR FLAGFISH EGGS

FIGURE 2. BIOASSAY APPARATUS

water was filtered. The filtered/sterilized samples were collected and counted for bacterial and viral populations. This test was repeated three times. Results (Table 1) show that the filtration system averaged about 74% removal of the two organisms.

The turbidity (NTU) (Table 2) of the filtered seawater was generally less than 10 NTU. The filtration system averaged 70% removal of particulates.

### Continuous-Flow Bioassay Tanks (Figure 2 B)

Ten bioassay tanks, constructed of 0.64-cm plexiglass, had dimensions of 60 cm long, 30 cm wide, and 40 cm deep. A 1.9-cm I. D., 9.3-cm high plexiglass standpipe was centered 6 cm from one end of each tank to give a working volume of 16.8 ℓ. A 0.32-cm plexiglass baffle 22 x 30 cm in size was placed 9 cm from the inlet side and 8 cm from the tank bottom to prevent short-circuiting and increase current flow for favorable aufwuchs growth. A flow rate of 100 mℓ/min provided a mean hydraulic residence time of 2.8 hr.

### Fish Cages and Aufwuchs Racks

To isolate fish from aufwuchs in the small-scale bioassay tanks, fish cages and aufwuchs racks were constructed. Fish cages were 25 cm long, 16 cm wide, and 10 cm deep and constructed from 2.5-mm mesh 316 stainless steel wire cloth. The aufwuchs racks were 29.3 cm long, 7 cm wide, and 1 cm high and were constructed of 1.1-cm plexiglass. Each rack held 7 growth units – roughened tygon tubing 5-cm long and 1.2 cm in diameter. The fish cages and aufwuchs racks were suspended 16 cm and 5 cm from the tank bottom, respectively, by 0.16-cm diameter stainless steel wire.

## FRESH WATER FISH AND APPARATUS

### Source

Fresh water was pumped to the Sanitary Engineering Research Laboratory (SERL) from a well located on the Richmond Field Station.

### Flagfish Stock

The lineage of the flagfish eggs was from an $F_1$ generation raised at SERL. Their parents were obtained from the Four-Star Fish Farm of Bradenton, FL. The number of $F_1$ males in the lineage was 4 and the number of females was 4–16. In each of 4 breeding aquaria there were 4 females and one male.

### Circulation System for Flagfish Egg Cups

The circulation method was changed from the previously-used pulley agitation system to an influent flow-through system (Figure 2C). The new method directed a portion of the tank influent into the egg cup. The influent

TABLE 1

BACTERIA AND VIRUS CONCENTRATIONS BEFORE AND AFTER FILTRATION

| Test No. | Organism | Initial Conc. Number/ $m\ell \times 10^3$ | Filtered Conc. Number/ $m\ell \times 10^2$ | Removal % |
|---|---|---|---|---|
| 1 | *E. coli* B | 4.3 | 9.3 | 78 |
| | Poliovirus | 1.8 | 5.5 | 69 |
| 2 | *E. coli* B | 4.3 | 15 | 65 |
| | Poliovirus | 2.8 | 5.0 | 82 |
| 3 | *E. coli* B | 9.1 | 24 | 74 |
| | Poliovirus | 2.6 | 6.3 | 76 |

TABLE 2

TURBIDITY OF SEA WATER BEFORE AND AFTER FILTRATION

| Test No. | Initial NTU | Filtered NTU | Removal % |
|---|---|---|---|
| 1 | 26 | 4 | 85 |
| 2 | 54 | 9 | 83 |
| 3 | 30 | 6 | 80 |
| 4 | 12 | 8 | 33 |
| 5 | 39 | 11 | 72 |

system consisted of a mixing chamber which received the flow of dilution water and WSF through a funnel. The mixing chamber was a 3.79-ℓ capacity wide-mouth jar and the funnel directed the two streams to the bottom of the jar. The liquid was siphoned from a point near the top of the jar to ensure that the two streams were well mixed before being transferred to the egg cups. The cups were each contained in 500-mℓ beakers and were positioned near the surface so that the incoming stream caused sufficient turbulence to the eggs to prevent fungus growth. The total flow into each tank via the mixing jar was 110 mℓ/min and of this approximately 60-72 mℓ/min was directed through the egg cups.

During the egg hatching period the eggs were observed daily through a magnifier and transferred into fresh egg cups. Used egg cups were immersed in boiling water for 1 hr for disinfection. The eggs were treated daily with malachite green to aid in combating fungus contamination.

### Rainbow Trout Stock

Eyed eggs of rainbow trout (Salmo gairdneri) were obtained from the Mount Lassen Trout Farm of Red Bluff, CA.

### Rainbow Trout Egg Baskets

Trout eggs were contained in 8-mesh, wire (0.071-cm diam.) baskets 25.4 cm by 25.4 cm square and 10.2 cm deep. The baskets were suspended to a depth of 5 cm in the continuous flow bioassay tanks. Circulation was provided by the influent streams of dilution water and WSF.

### Solubilizers, Distribution System, Bioassay Tanks

This apparatus was the same as used in previous work (November 1977 Second Annual Report AMRL-TR-77-54). Fuel in the solubilizers was replenished once per week.

### Golden Shiners (Notemigonus chrysoleucas)

Golden shiners were obtained from the Sierra Bait Company of Sacramento, CA, and were used for range-finding purposes.

## SHALE JP-8 ACUTE FRESH WATER BIOASSAYS

### INTRODUCTION

Prior to the conduct of chronic studies with rainbow trout, the test species golden shiner was subjected to a series of short-term bioassays. The purpose was two-fold: (1) to determine the acute toxicity of the WSF of shale JP-8 for comparison with the results obtained for other hydrocarbon fuels, and (2) to serve as range-finding tests to establish the proper concentrations for examination in the subsequent chronic study.

The choice of golden shiner as the test species was made to enable the comparison mentioned above and because of the availability of proper size fish to conduct acute bioassays.

ACUTE STATIC 96-hr BIOASSAY

Ten golden shiners each were placed in 18.9-ℓ jars containing duplicates of 0, 32, 50, 63, 79, and 100% WSF of shale JP-8. These solutions were renewed every 24 hr. The fish had an average forked length of 4.4 ± 0.4 cm and an average wet weight of 0.84 ± 0.3 g. Each jar contained 15 ℓ dilution water (well water) maintained at 21 ± 1°C and aerated at 17 ± 3 bubbles per min. Dissolved oxygen (DO) was 7.8 ± 0.6 mg/ℓ and the pH was 7.5 ± 0.2 during the course of the study.

Results of fuel concentration measurements and fish survival are presented in Table 3. The average percent survival indicated that the 96-hr LC 50 of WSF of shale JP-8 was between 2.91 and 3.77 mg/ℓ, based on initial concentration measurements.

TABLE 3

GOLDEN SHINER SURVIVAL IN 96-hr ACUTE STATIC EXPOSURE TO WSF OF SHALE JP-8

| WSF of Shale JP-8 | | | | | | |
|---|---|---|---|---|---|---|
| Dilution of WSF % | Measured Initial Conc. mg/ℓ | Measured After 24 hr mg/ℓ | Fish Survival, Number After Hour: 24 | 48 | 72 | 96 |
| | | Series A | | | | |
| 0 | 0 | 0 | 10 | 10 | 10 | 10 |
| 32 | 0.74 | 0.46 | 10 | 10 | 10 | 10 |
| 50 | 1.88 | 1.27 | 10 | 10 | 10 | 9 |
| 63 | 2.43 | 1.69 | 10 | 10 | 10 | 10 |
| 79 | 2.93 | 2.01 | 10 | 10 | 10 | 9 |
| 100 | 3.88 | 2.54 | 7 | 3 | 3 | 3 |
| | | Series B | | | | |
| 0 | 0 | 0 | 10 | 10 | 10 | 10 |
| 32 | 0.88 | 0.45 | 10 | 10 | 10 | 10 |
| 50 | 1.88 | 0.85 | 10 | 10 | 10 | 10 |
| 63 | 2.47 | 1.57 | 10 | 10 | 9 | 9 |
| 79 | 2.89 | 2.01 | 10 | 10 | 10 | 9 |
| 100 | 3.66 | 2.81 | 9 | 7 | 6 | 6 |

The concentration of shale JP-8 in each dilution was measured at the beginning and end of the 24-hr fuel renewal interval. Table 4 presents the average of dilutions and the GC measurements except for the lowest (32%) dilution. Excluding this value, the fuel lost by volatility and biodegradation in a 24-hr period is between 21 and 29% of the initial concentration.

TABLE 4

AVERAGE WSF OF SHALE JP-8 CONCENTRATION
(Combined Results of Series A and B)

| Volumetric Dilutions of WSF of Shale JP-8 % | Measured Concentration | | Loss During 24 hr % |
|---|---|---|---|
| | Initial mg/ℓ | After 24 hr mg/ℓ | |
| 0 | 0 | 0 | 0 |
| 32 | 0.81 | 0.46 | 9 |
| 50 | 1.88 | 1.06 | 21 |
| 63 | 2.45 | 1.62 | 22 |
| 79 | 2.91 | 2.01 | 24 |
| 100 | 3.77 | 2.68 | 29 |

Fish behavior was observed daily before and after renewal of the solutions. Ten minutes after exposure to renewed fuel solution the fish in the 0 and 32% concentrations appeared normal, but those in the 50% and 63% concentrations were dark colored and swam between the middle and surface of the water; the fish in the 79% and 100% concentrations were dark colored and remained at the water surface.

Three hours after exposure, the behavior became more extreme. Fish in the controls appeared normal; the fish in the 32% concentration were dark with one fish remaining at the water surface; the fish in the 50% and 63% concentrations were dark and all were at the surface. One appeared to be moribund. The fish in the 79% and 100% concentrations were dark and swam at a 45 deg. angle to the water surface. Seven fish appeared moribund.

This behavior persisted throughout the experiment with the fish exposed to the higher fuel concentrations demonstrating the greater stress symptoms. Only the fish in the lowest (32%) fuel concentration recovered and appeared normal each day before the fuel was renewed.

## EIGHTEEN-DAY CONTINUOUS-FLOW BIOASSAY

Golden shiners were exposed to the WSF of shale JP-8 in the stainless steel continuous-flow tanks over an 18-day period at nominal hydraulic residence time of 6 hr and in a liquid volume of 80 ℓ. Twenty-one fish (average forked length, 4.4 ± 0.4 cm; average wet weight, 0.84 ± 0.3 g) were maintained in each tank. Nominal duplicate percentage dilutions of WSF of shale JP-8 were 0, 13, 25, 42, and 79% in RFS well water. The tanks were unaerated; DO remained above 60% saturation throughout the study. The pH was 7.4 ± 1.0 and the average temperature was 19.6 ± 0.7°C. WSF and dilution water flow rates were adjusted daily. The tanks and the

solubilizer were cleaned weekly. The fish were fed twice each week.

No difference in mortality was observed between the controls and the 42% dilution (Table 5); in the 79% dilution there was approximately 50% mortality by Day 18. From the observed trend, we speculated that no fish would survive at this concentration during a long-term partial chronic bioassay.

TABLE 5

SURVIVAL OF GOLDEN SHINERS IN AN 18-DAY CONTINUOUS-FLOW BIOASSAY

| Dilution of WSF of Shale JP-8 % | Measured Shale JP-8 Concentration, mg/ℓ | | | Fish Survival, % | |
|---|---|---|---|---|---|
| | Day 1* | Day 7** | Mean | Day 15 | Day 18 |
| Bank A | | | | | |
| 0 | 0 | 0 | 0 | 95 | 95 |
| 13 | 0.15 | 0.08 | 0.12 | 90 | 90 |
| 25 | 0.30 | 0.26 | 0.28 | 86 | 86 |
| 42 | 0.57 | 0.43 | 0.50 | 95 | 95 |
| 79 | 2.13 | 1.76 | 1.94 | 57 | 47 |
| Bank B | | | | | |
| 0 | 0 | 0 | 0 | 90 | 90 |
| 13 | 0.17 | 0.14 | 0.16 | 95 | 95 |
| 25 | 0.36 | 0.31 | 0.31 | 90 | 90 |
| 42 | 0.62 | 0.53 | 0.53 | 95 | 95 |
| 79 | 1.99 | 1.72 | 1.72 | 81 | 57 |
| 100 | 3.99 | 4.08 | 4.04 | | |

* Fuel age = 1 day

** Fuel age = 7 days

Fish behavior supported this premise. There was no difference in fish behavior between the control and the 13% dilution. However, stress symptoms became increasingly pronounced at higher fuel concentrations and with length of exposure. Slight stress symptoms, dark coloration, and

slower pursuit of food were noted in the fish exposed to 25% and 42% dilutions of WSF. At the 79% dilution, the fish did not eat, were very dark, and about half of them were swimming at an angle 45 deg. to the water surface.

Based on these results, we concluded that the 18-day LC 50 was approximately a 79% dilution of WSF of shale JP-8 which is equivalent to approximately 1.83 mg/ℓ. From this information the nominal percent dilutions for the partial chronic bioassay on rainbow trout were selected as 0, 6, 13, 25, and 50%.

The measured WSF concentrations were considerably lower than the nominal concentrations. Assuming that the 100% WSF for shale JP-8 was 4.04 mg/ℓ, the dilutions of 13, 25, and 42% should have produced about four times the observed concentrations in mg/ℓ. This difference was attributed largely to biodegradation since the WSF does not appear to be highly volatile, as shown by purging results (see Materials and Methods).

A yellow color appeared in the tanks and became more intense at higher WSF concentrations. The color became more vivid with increasing fuel age suggesting that it may be related to fuel biodegradation (see Appendix 4).

## PARTIAL CHRONIC BIOASSAY OF WSF OF SHALE JP-8 USING RAINBOW TROUT

### INTRODUCTION

This bioassay was conducted to determine the no-effect level of the WSF of shale JP-8 on rainbow trout survival, growth, and egg hatching. In addition, the accumulation of fuel in fish tissue after long-term exposure was measured.

### PROCEDURE

Based on the results of the 18-day continuous, acute bioassay on golden shiners, a range of fuel concentrations was selected which was expected to bracket the no-effect level for lethality. The duplicate dilutions of WSF of shale JP-8 studied were 0, 6, 13, 25, and 50% of the solubilizer effluent.

The study was initiated with 10 egg baskets each containing 324 eyed eggs of rainbow trout. Each egg basket was placed in a stainless steel tank. The conditions were identical to those used in previous cold water studies (November 1979 Fourth Annual Report AMRL-TR-79-70). The tanks each contained 80 ℓ of liquid and received a flow of 220 mℓ/min to give a nominal hydraulic residence time of 6 hr. The water was maintained at approximately 15°C; no aeration was provided.

Parameters measured daily were temperature and flow rates of solubilizer product of shale JP-8 and dilution water. The pH, DO, and shale JP-8 concentration were routinely measured weekly; well water chemical analysis was performed twice. During critical periods of the study the DO was measured daily.

Fish were fed 8 times per day. Maintenance involved daily removal of excess food, twice-weekly cleaning of tank sides, and once-weekly cleaning

of tank contents by recirculating them through a synthetic wool filter.

To control fungus growth the eggs and hatched fry were treated daily for two weeks by adding 10 drops of an 0.1 mg/ℓ solution of Malachite Green to each tank.

## EXPERIMENTAL CONDITIONS

The mean water temperature was 14.5 ± 1.2°C; there was no variation between tanks.

Mean pH and DO values are presented in Table 6. There was a pH difference of 0.10 between dilution water and solubilizer product (100% WSF of shale JP-8). The mean pH of the individual bioassay tanks was in the range 7.57–7.63.

There was a slight difference in DO between tanks which was related to the effect of fuel on fish. At the two highest fuel concentrations the mean DO ranged from 9.0–9.2 mg/ℓ and at the three lowest fuel concentrations the DO was 8.9 mg/ℓ. At lower fuel concentrations fish growth was greater – hence their metabolic activity was greater. As soon as there was a noticeable decline in DO (0.1 mg/ℓ) below 8 mg/ℓ the fish were thinned.

The dilution water characteristics (Table 7) are based on three sets of analyses.

## MEASURED WSF OF SHALE JP-8 CONCENTRATIONS

The WSF of shale JP-8 concentrations in the exposure tanks were measured weekly by the pentane extraction GC method. Results are presented in Table 8. The mean of duplicate measurements of the solubilizer effluent (100% WSF of shale JP-8) was obtained each sampling day. The mean of these means was 3.48 mg/ℓ; the standard deviation of the individual standard deviations was 0.20 mg/ℓ. The RSD was 5.7%.

The controls (Tanks 1A and 1B) are omitted from Table 8 because they contain no WSF. The 6% tanks (2A and 2B) contained concentrations of 0.13 and 0.12 mg/ℓ WSF, respectively. Based on the 100% WSF value of 3.48 mg/ℓ, the measured percent WSF in these tanks was 3.7 and 3.4%, respectively, indicating a loss of 38% and 42%, respectively. Similarly, the measured concentrations in Tanks 3, 4, and 5 were compared with the nominal exposure level and indicated a loss of 25–31%. This loss may be due in part to calibration error, but volatility and/or biodegradation are also responsible (see 24-hr differences in acute study).

These results generally indicated increasing losses at progressively lower WSF concentrations. This may be attributable to the effect of the fuel on fish growth and behavior. At the lower fuel concentrations the fish are larger and more active. Fish activity contributes to turbulence which enhances volatility loss; biodegradation may be enhanced because the amount of waste products present in the tanks occupied by larger fish supports higher microbial populations.

TABLE 6

DISSOLVED OXYGEN AND pH OF EXPOSURE TANK WATERS DURING CHRONIC RAINBOW TROUT BIOASSAY

| Source | pH ± s | DO ± s mg/ℓ |
|---|---|---|
| Dilution water | 7.49 ± 0.09 | 8.9 ± 0.5 |
| Solubilizer product | 7.59 ± 0.09 | 9.2 ± 0.5 |
| Bank A | | |
| 1 | 7.60 ± 0.08 | 8.9 ± 0.9 |
| 2 | 7.60 ± 0.07 | 8.9 ± 0.8 |
| 3 | 7.61 ± 0.07 | 8.9 ± 0.8 |
| 4 | 7.61 ± 0.06 | 9.1 ± 0.8 |
| 5 | 7.61 ± 0.05 | 9.2 ± 0.7 |
| Bank B | | |
| 1 | 7.57 ± 0.08 | 8.8 ± 0.8 |
| 2 | 7.61 ± 0.05 | 8.9 ± 0.8 |
| 3 | 7.63 ± 0.06 | 8.9 ± 0.9 |
| 4 | 7.63 ± 0.06 | 9.0 ± 0.8 |
| 5 | 7.63 ± 0.05 | 9.2 ± 0.8 |

TABLE 7

DILUTION WATER CHARACTERISTICS DURING CHRONIC RAINBOW TROUT BIOASSAY

| Parameter | Measurement ± s |
|---|---|
| Total Solids, mg/ℓ | 683 ± 100 |
| Total Dissolved Solids, mg/ℓ | 626 ± 75 |
| Total Hardness (as $CaCO_3$), mg/ℓ | 312 ± 8 |
| pH | 7.5 |
| Chloride, mg/ℓ | 84 ± 3 |
| Sulfate, mg/ℓ | 44 ± 10 |

TABLE 8

WSF OF SHALE JP-8 CONCENTRATIONS DURING PARTIAL CHRONIC RAINBOW TROUT BIOASSAY (mg/ℓ)

| Day | Fuel Age days | 100% WSF of Shale JP-8 mg/ℓ ± s * | Bank A | | | | Bank B | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Tank 2 6% | Tank 3 13% | Tank 4 25% | Tank 5 50% | Tank 2 6% | Tank 3 13% | Tank 4 25% | Tank 5 50% |
| 0 | 1 | 4.11 ± 0.08 | 0.17 | 0.45 | 0.81 | 1.70 | 0.12 | 0.54 | 0.81 | 1.68 |
| 7 | 7 | 3.47 ± 0.28 | 0.12 | 0.32 | 0.50 | 1.11 | 0.16 | 0.51 | 0.87 | 1.12 |
| 13 | 7 | – | 0.13 | 0.35 | 0.62 | 1.69 | 0.11 | 0.23 | 0.85 | 1.44 |
| 20 | 7 | 3.83 ± 0.08 | 0.04 | 0.19 | 0.34 | 1.64 | 0.07 | 0.41 | 0.41 | 1.53 |
| 27 | 7 | 3.18 | 0.06 | 0.16 | 0.39 | 0.94 | 0.03 | 0.17 | – | 0.94 |
| 34 | 7 | 3.69 ± 0.67 | 0.15 | 0.32 | 0.35 | 0.99 | 0.10 | 0.25 | 0.36 | 0.98 |
| 46 | 5 | 3.64 ± 0.30 | 0.18 | 0.20 | 0.62 | 0.79 | 0.06 | 0.31 | – | 1.02 |
| 49 | 1 | 3.62 ± 0.34 | 0.17 | 0.32 | 0.93 | 1.48 | 0.09 | 0.48 | 0.64 | 1.31 |
| 61 | 6 | 3.24 ± 0.21 | 0.21 | 0.41 | 0.75 | 1.35 | 0.17 | 0.41 | 0.76 | 1.23 |
| 69 | 7 | 3.59 ± 0.09 | 0.15 | 0.31 | 0.60 | 0.99 | 0.18 | 0.28 | 0.71 | 1.23 |
| 77 | 7 | 3.67 | 0.07 | 0.21 | 0.58 | 1.28 | 0.21 | 0.20 | 0.65 | 1.15 |
| 82 | 5 | 2.92 | 0.08 | – | 0.56 | 1.16 | 0.08 | 0.15 | 0.60 | 1.24 |
| 90 | 6 | 3.17 ± 0.06 | 0.10 | 0.32 | 0.68 | 1.24 | 0.05 | 0.33 | 0.55 | 1.13 |
| 97 | 6 | 3.03 ± 0.02 | 0.17 | 0.47 | 0.72 | 1.19 | 0.18 | 0.38 | 0.68 | 1.20 |
| 104 | 6 | 2.93 ± 0.01 | – | 0.36 | 0.62 | 1.23 | 0.17 | 0.14 | 0.59 | 1.06 |
| Mean ± s | | 3.48 ± 0.20 | 0.13 ± 0.05 | 0.31 ± 0.10 | 0.60 ± 0.17 | 1.25 ± 0.28 | 0.12 ± 0.06 | 0.32 ± 0.13 | 0.65 ± 0.16 | 1.22 ± 0.21 |
| Measured % WSF of Shale JP-8 | | | 3.7 | 8.9 | 17.2 | 35.9 | 3.4 | 9.2 | 18.7 | 35.1 |
| % Loss of WSF (Volatility and Biodegradation) | | | 38 | 31 | 31 | 28 | 42 | 29 | 25 | 30 |

* N = 2

A similar phenomenon occurred on a temporal basis. Results show a decline in fuel concentration as the experiment progresses and the fish grow larger.

## EGG HATCHING

Table 9 shows the cumulative number and percent of eggs hatching. Eggs hatched in 4 to 9 days after exposure to WSF of shale JP-8. Hatching was in the range of 91-94%; the total percent hatched appeared to be unaffected by any of the levels of WSF of shale JP-8.

WSF of shale JP-8 concentrations shown in Table 9 are the means of 6 measurements taken during the initial stage of the study (between Days 1 and 34); and indicate the following approximate exposure concentrations: 0, 0.10, 0.32, 0.60, and 1.29 mg/$\ell$ WSF of shale JP-8 for the 0, 6, 13, 25, and 50% dilutions, respectively.

Table 9 indicates an acceleration in hatching rate after Day 5 for WSF of shale JP-8 concentrations of $\geq$ 0.60 mg/$\ell$. Acceleration of rainbow trout egg hatching was previously observed with WSF of JP-8 at concentrations $\geq$ 2.1 mg/$\ell$ and with the WSF of JP-4 at concentrations $\geq$ 1.7 mg/$\ell$ (November 1979 Fourth Annual Report AMRL-TR-79-70).

## FISH SURVIVAL

A large initial fish population was placed in each exposure tank to provide sufficient numbers for statistical evaluation and also to allow the use of thinning procedures to measure growth rate. Final survival data and thinning measures are presented in Table 10. Fish were thinned on Days 25, 47, 60, 73, and 94. The decision to thin was based on DO measurements in the tanks. When the DO dropped by 0.5 to 1.5 mg/$\ell$ in the tanks containing the lower fuel concentrations the population was thinned.

The mean percent fish mortality is presented in Figure 3. It is concluded that the no-effect level with respect to survival was between 0.13 and 0.32 mg/$\ell$.

## FISH GROWTH

The effect of the fuel on fish growth rate was evaluated by measuring the length and weight of fish removed from the tanks to thin the population. The detailed data for each bank of tanks are tabulated in Appendix 1. Mean fish length is plotted in Figure 4; mean fish weight is plotted in Figure 5. Both the weight and length measurements show decreased growth at each successively higher fuel concentration. The decrease became more pronounced between Days 94 and 119. The difference in length between the control fish and fish in the lowest fuel concentration (0.13 mg/$\ell$) was statistically significant by Day 119. ($T_{calculated}$ = 12.5, $T_{tabular}$ = 6.9 at the 1% level.)

TABLE 9

HATCHING OF RAINBOW TROUT EGGS IN WSF OF SHALE JP-8

| Tank | WSF of Shale JP-8 Dilution of WSF % | WSF of Shale JP-8 Concn. mg/ℓ ±s | Original Number of Eggs | Cumulative Number of Trout Hatched on Day: 4 | 5 | 6 | 7 | 8 | 9 | Total Hatched % |
|---|---|---|---|---|---|---|---|---|---|---|
| Bank A | | | | | | | | | | |
| 1 | 0 | 0 | 324 | 5 | 183 | 295 | 306 | 306 | 306 | 94 |
| 2 | 6 | 0.11 ± 0.05 | 324 | 7 | 194 | 291 | 293 | 294 | 296 | 91 |
| 3 | 13 | 0.29 ± 0.10 | 324 | 16 | 179 | 277 | 294 | 295 | 295 | 91 |
| 4 | 25 | 0.50 ± 0.18 | 324 | 7 | 230 | 302 | 306 | 307 | 307 | 95 |
| 5 | 50 | 1.34 ± 0.37 | 324 | 31 | 217 | 299 | 302 | 302 | 302 | 93 |
| Bank B | | | | | | | | | | |
| 1 | 0 | 0 | 324 | 5 | 132 | 227 | 293 | | | 90 |
| 2 | 6 | 0.09 ± 0.04 | 324 | 5 | 159 | 290 | 297 | | | 92 |
| 3 | 13 | 0.35 ± 0.16 | 324 | 8 | 164 | 295 | 295 | | | 91 |
| 4 | 25 | 0.71 ± 0.32 | 324 | 6 | 271 | 299 | 303 | | | 94 |
| 5 | 50 | 1.24 ± 0.35 | 324 | 1 | 241 | 295 | 298 | | | 92 |
| Banks A and B | | | | | | | | | | |
| 1 | 0 | 0 | 648 | 10 | 315 | 522 | 599 | 599 | 599 | 92 |
| 2 | 6 | 0.10 ± 0.05 | 648 | 12 | 353 | 581 | 590 | 591 | 593 | 92 |
| 3 | 13 | 0.32 ± 0.13 | 648 | 24 | 343 | 572 | 589 | 590 | 590 | 91 |
| 4 | 25 | 0.60 ± 0.26 | 648 | 13 | 501 | 601 | 609 | 610 | 610 | 94 |
| 5 | 50 | 1.29 ± 0.36 | 648 | 32 | 458 | 594 | 600 | 600 | 600 | 93 |

N = 6

TABLE 10

SURVIVAL AND THINNING OF RAINBOW TROUT DURING CHRONIC EXPOSURE TO WSF OF SHALE JP-8

| Dilution of WSF % | WSF of Shale JP-8 Concn. mg/ℓ | Initial Number of Eggs Added Day 0 | Number of Eggs Hatched Day 7 | Number of Survivors — Thinned to Number Specified | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Day 25 | Day 47 | Day 60 | Day 73 | Day 94 | Day 119 |
| Bank A | | | | | | | | | |
| 0 | 0 | 324 | 306 | 282 → 230 | 218 → 175 | 169 → 124 | 124 → 80 | 78 → 31 | 31 |
| 6 | 0.13 | 324 | 293 | 277 → 230 | 216 → 175 | 169 → 124 | 123 → 80 | 74 → 30 | 29 |
| 13 | 0.31 | 324 | 294 | 270 → 230 | 211 → 175 | 164 → 124 | 122 → 80 | 76 → 30 | 25 |
| 25 | 0.60 | 324 | 306 | 273 → 230 | 198 → 175 | 167 → 124 | 120 → 80 | 74 → 30 | 27 |
| 50 | 1.25 | 324 | 302 | 252 → 230 | 68 → 68 | 44 → 44 | 41 → 41 | 31 → 31 | 27 |
| Bank B | | | | | | | | | |
| 0 | 0 | 324 | 293 | 259 → 230 | 214 → 175 | 173 → 124 | 119 → 80 | 77 → 30 | 28 |
| 6 | 0.12 | 324 | 297 | 282 → 230 | 212 → 175 | 171 → 124 | 121 → 80 | 77 → 31 | 31 |
| 13 | 0.32 | 324 | 295 | 267 → 230 | 215 → 175 | 171 → 124 | 122 → 80 | 72 → 30 | 28 |
| 25 | 0.65 | 324 | 303 | 273 → 230 | 199 → 175 | 165 → 124 | 123 → 80 | 68 → 30 | 27 |
| 50 | 1.22 | 324 | 298 | 261 → 230 | 83 → 83 | 58 → 58 | 54 → 54 | 41 → 41 | 34 |

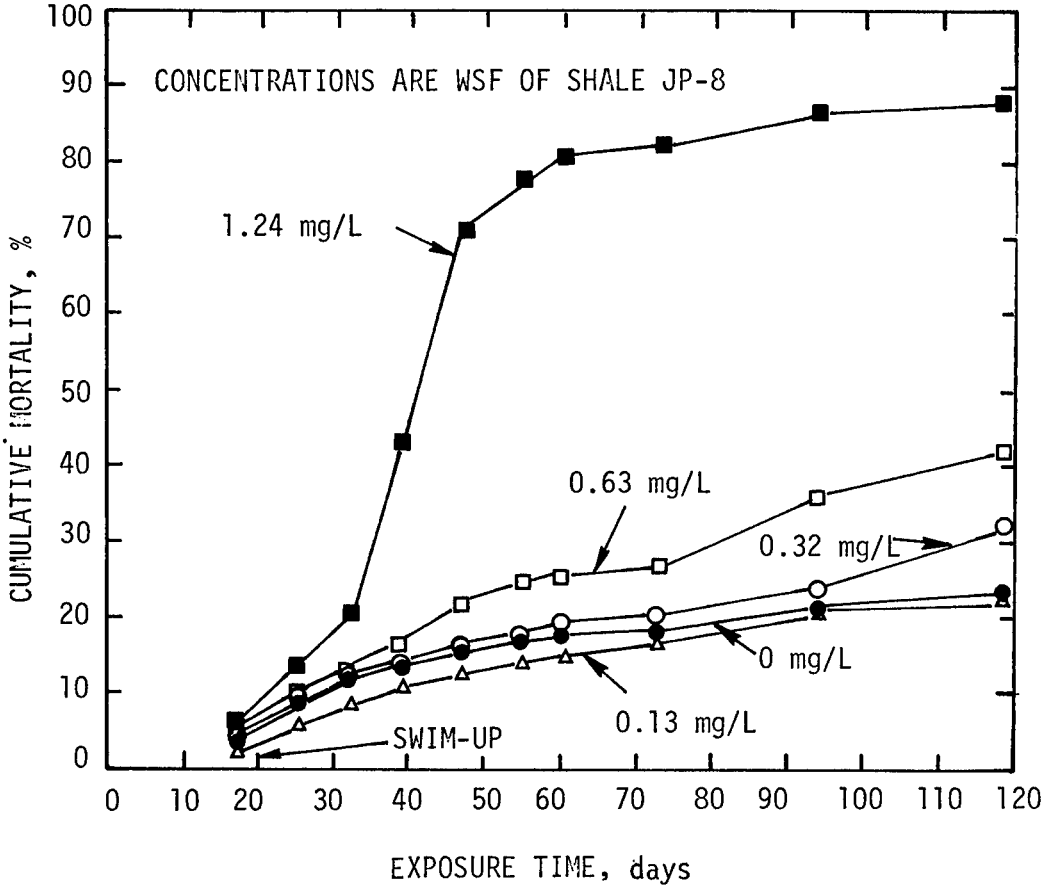



FIGURE 3. CUMULATIVE MORTALITY IN RAINBOW TROUT EXPOSED TO WSF OF SHALE JP-8

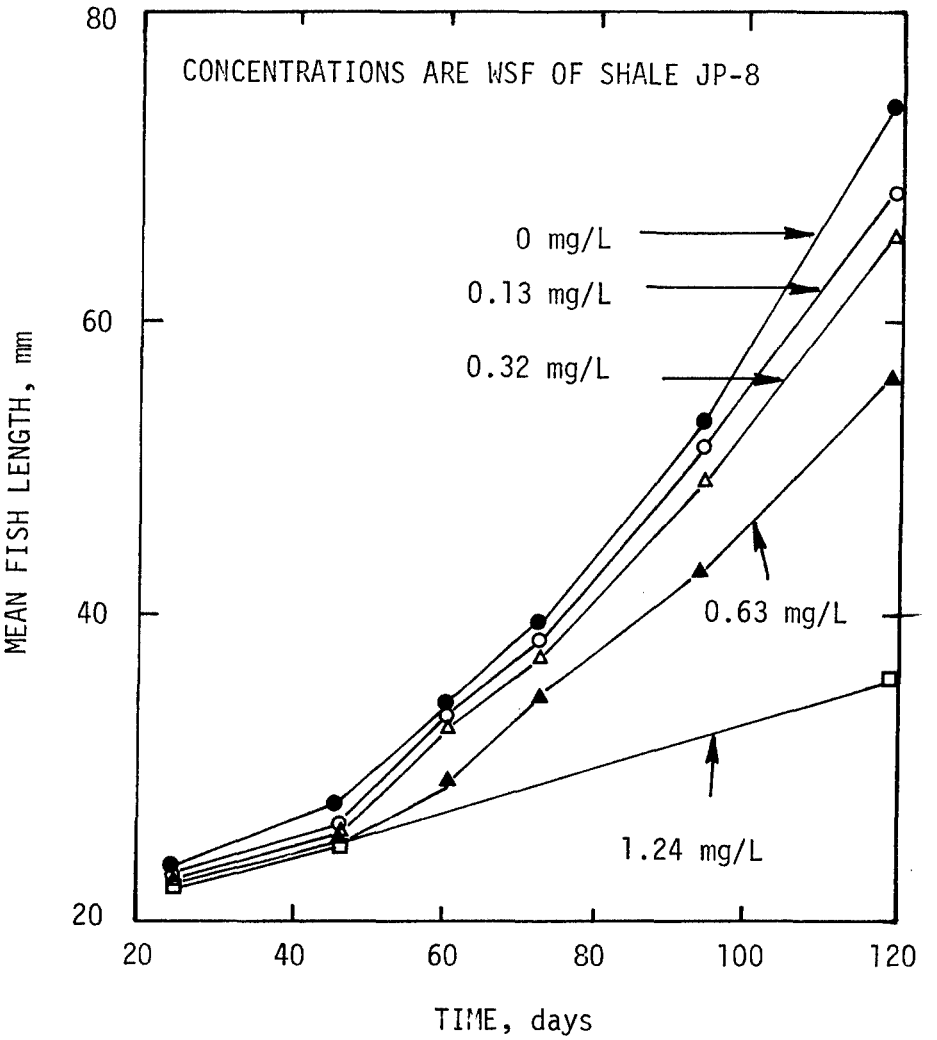



FIGURE 4. EFFECT OF WSF OF SHALE JP-8 ON RAINBOW TROUT LENGTH.

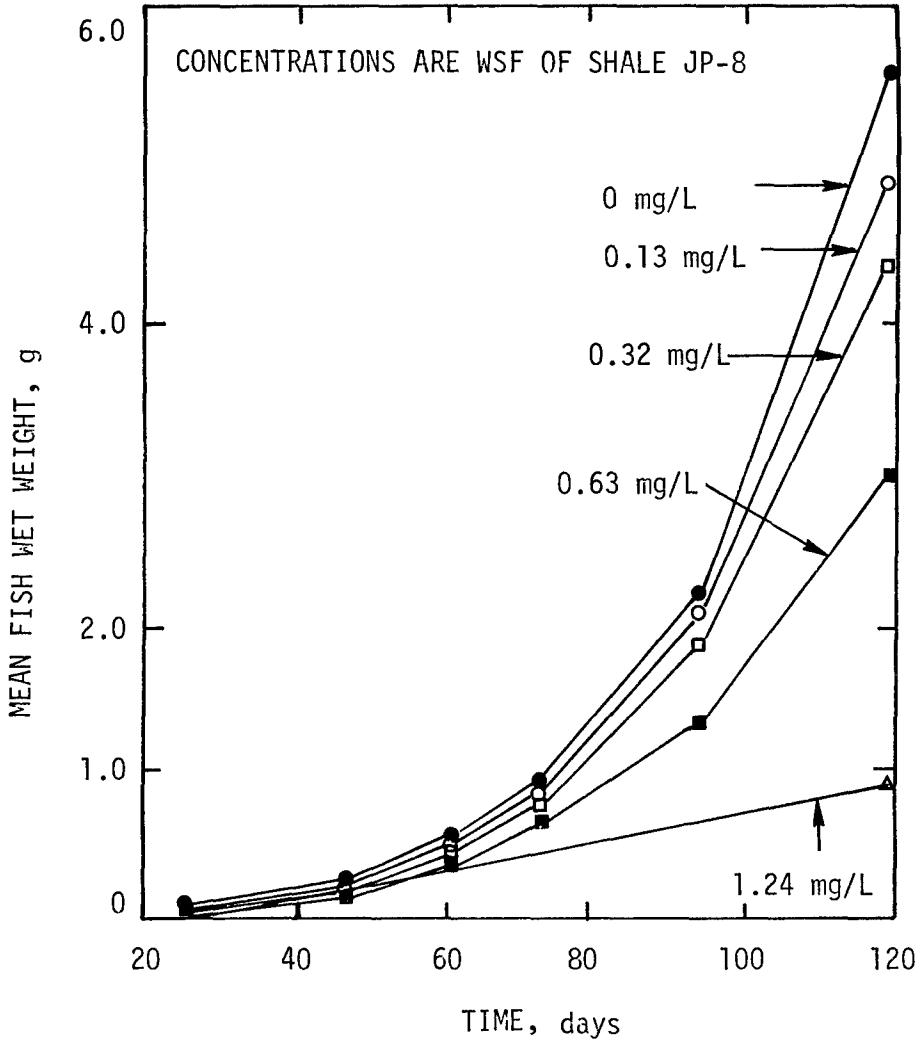



FIGURE 5. EFFECT OF WSF OF SHALE JP-8 ON RAINBOW TROUT WET WEIGHT.

The F-test using length data of all duplicate sets from Bank A + B on Day 119 indicates there is a high probability (greater than 99%) of there being a difference in length due to concentration ($F_{calculated} = 182$, $F_{tabular\ (4,282)} = 7$ at the 1% level). The two banks show excellent replication, since there is no significant difference between A and B ($F_{calculated} = 0.58$, $F_{tabular\ (1,285)} = 3.9$ at the 5% level).

The results show that the no-effect level with respect to growth was not elicited, but is in the range of 0 to $0.13 \pm 0.06$ mg/ℓ. Regression analysis (Figure 6) indicates that the maximum predicted no-effect level is 0.06 mg/ℓ.

## FUEL ACCUMULATION

The accumulation of WSF of shale JP-8 in rainbow trout was analyzed at the conclusion of the bioassay. After 119 days of exposure the fish were removed from the bioassay tanks, measured for length and weight, and stored in a freezer until they could be processed for fuel accumulation measurement. Dissection was performed on some fish to determine fuel accumulation in the liver, muscle, carcass, and viscera. Other fish were reserved for whole-body analysis.

The analytical procedure was to grind the fish or individual tissues in a Virtis tissue grinder with anhydrous $Na_2SO_4$ and pentane. The pentane extract was filtered through a florosil column and concentrated to 1.0 mℓ before aliquots were injected into the GC.

Results, presented in Table 11, indicate a progressive accumulation of fuel in whole-body tissue of fish exposed to successively higher WSF of shale JP-8 concentrations. Percent relative standard deviations were 40.7, 43.0, 31.3, and 15.2 at WSF of shale JP-8 concentrations of 0.13, 0.32, 0.63, and 1.2 mg/ℓ, respectively. One factor that reduced the analytical accuracy (particularly in the lower-level exposure tanks) was the high background in the control fish. The value of 25 mg/kg in the control reflected organics in the fish tissue such as lipids that are extractable in pentane.

Specific tissue analyses did not yield very meaningful results. There was no indication of accumulation in the liver once the control background was subtracted from the accumulation values. Two of the values from the 0.32 mg/ℓ exposure (55 mg/kg and 54 mg/kg) are inexplicably low. Muscle tissue appeared to show an increase in accumulation with increasing aqueous shale JP-8 concentration.

The accumulation ratio in the whole body (Table 12) is the net accumulation obtained by subtracting the control and dividing by the aqueous fuel concentration. The ratio is in the range of 51 to 93 with a mean of 72 — a range similar to that found for JP-8 in rainbow trout (63 to 112). Both of these fuels were accumulated to a lesser degree than WSF of JP-4 which had a mean accumulation ratio of 170 in rainbow trout.

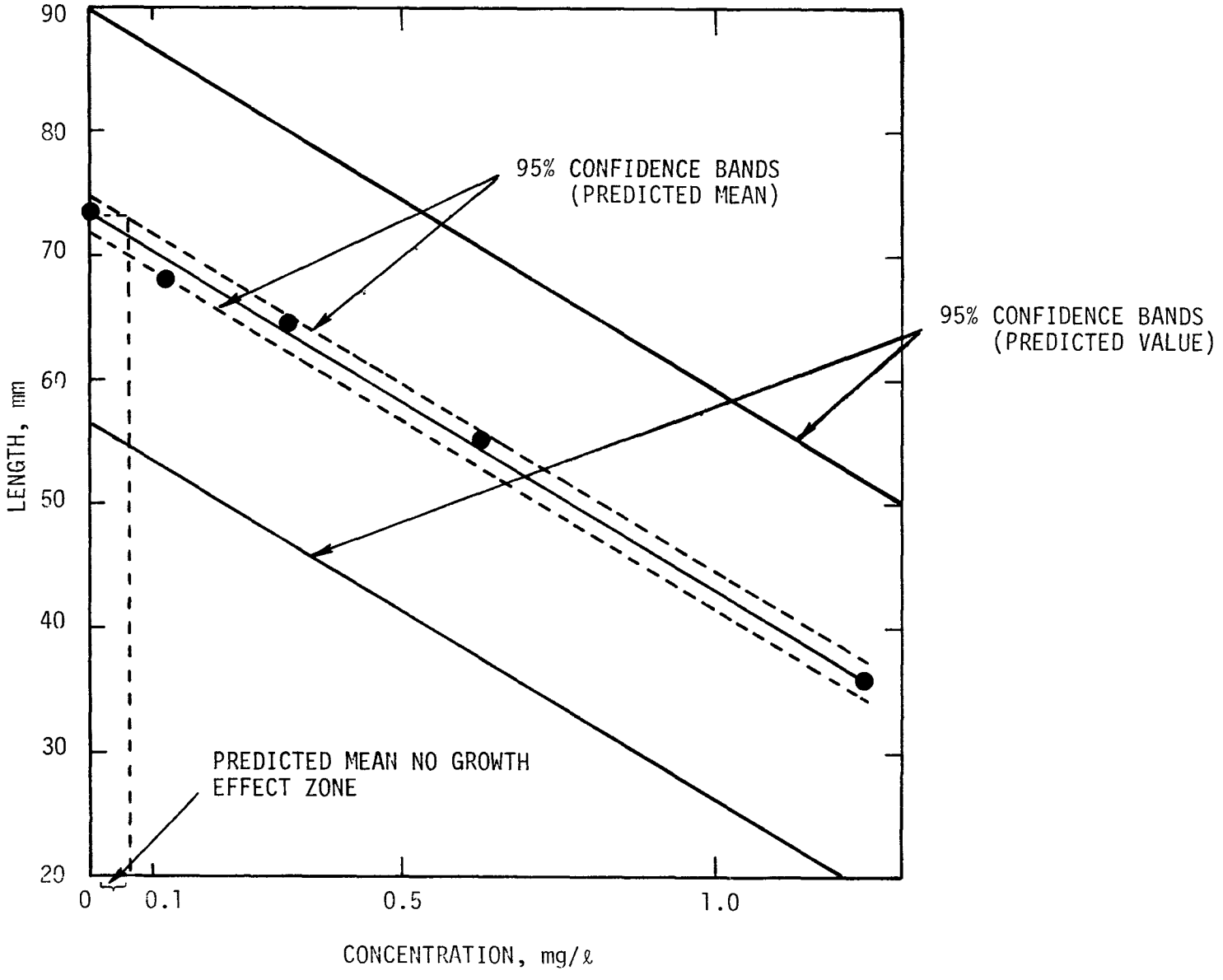



FIGURE 6. REGRESSION ANALYSIS OF MEAN RAINBOW TROUT FRY LENGTH (BANKS A AND D) ON DAY 119

TABLE 11

ACCUMULATION OF WSF OF SHALE JP-8 IN RAINBOW TROUT AFTER 119 DAYS OF EXPOSURE

(mg/kg)

| Tissue | Accumulation at Indicated Exposure Concentrations | | | | |
|---|---|---|---|---|---|
| | 0 mg/ℓ | 0.13 mg/ℓ | 0.32 mg/ℓ | 0.63 mg/ℓ | 1.24 mg/ℓ |
| Whole Body | 25 | 22 | 56 | 100 | 78 |
| | | 21 | 50 | 119 | 108 |
| | | 47 | 26 | 52 | 88 |
| | | 46 | 82 | 93 | 81 |
| | | 25 | | 83 | |
| | | | | 55 | |
| Mean ± s | | 32.2 ± 13.1 | 53.5 ± 23.0 | 83.7 ± 26.2 | 88.8 ± 13.5 |
| Carcass and Viscera | 24 | 17 | 29 | | |
| | 44 | 32 | 38 | | |
| | | | 61 | | |
| Mean ± s | 34.0 ± 14.1 | 24.5 ± 10.6 | 42.7 ± 16.5 | | |
| Muscle | 10 | 18 | 81 | | |
| | | 24 | | | |
| Liver | 241 | 208 | 272 | | |
| | 238 | 200 | 55 | | |
| | | | 54 | | |

TABLE 12

ACCUMULATION RATIO OF WSF OF JP-8 IN RAINBOW TROUT WHOLE BODY

| WSF of JP-8 Concentration, mg/ℓ | Accumulation | | Accumulation Ratio |
|---|---|---|---|
| | Total mg/kg | Net mg/kg | Net mg/kg / mg/ℓ |
| 0 | 25 | 0 | - |
| 0.13 | 32 | 7.2 | 55 |
| 0.32 | 54 | 28 | 89 |
| 0.63 | 84 | 59 | 93 |
| 1.2 | 89 | 54 | 51 |
| Mean: | | | 72 |

## CHRONIC BIOASSAY - WSF OF SHALE JP-8 TO FLAGFISH

### INTRODUCTION

A 148-day long-term bioassay was conducted to determine the chronic toxicity of the WSF of shale JP-8 to flagfish. Toxicity was assessed by the effect of WSF on egg hatching, fry growth and development, fry survival, and reproductive ability. Fuel accumulation also was to be studied, but the pentane extracts of fish tissue were accidently evaporated to dryness.

### PROCEDURE

The procedure was similar to that utilized in previous flagfish bioassays with the exception of the water circulation system provided for the egg cups (described in detail in Materials and Methods). Another departure from the usual work plan was the inclusion of an assessment of the reproductive ability of the fish subjected to long-term fuel exposure.

Based on preliminary range-finding bioassays, the following duplicate percent dilutions of WSF of shale JP-8 were studied: 0%, 6%, 13%, 25%, and 50%.

The lineage of the flagfish eggs was from an $F_1$ generation raised at SERL from 4 males and 4–16 females. The study was initiated with 166 eggs in each of 10 egg cups. At the time of exposure to fuel the eggs apportioned to each cup included 128 eggs which were 6–30 hr old and 38 eggs which were 30–54 hr old.

## EXPERIMENTAL CONDITIONS

The dilution water quality was characterized on 10 occasions separated by minimum intervals of one week. The results are presented in Table 13. The data show that the water (a groundwater) was of moderate TDS (540 mg/ℓ) and fairly high hardness (>300 mg $CaCO_3$/ℓ).

The temperature, pH, and DO in the bioassay tanks are reported in Table 14. Results are based on daily measurements taken throughout the study except for the temperature readings which represented the mean from Day 16 to Day 148. During the first 16 days room heaters could only raise the temperature of the cold water to 19.0 ± 0.9°C. To achieve the desired temperature level of 22°C, 10 aquarium heaters were installed in the dilution water head tank. For the remainder of the study the temperature of the tanks was in the range 22.2–22.3°C.

The DO in all tanks was maintained at a satisfactory level. At increasingly higher fuel concentrations there was a decrease in DO attributable to biodegradation of fuel. Biodegradation of fuel was observed in a previous study (November 1977 Second Annual Report AMRL-TR-77-54). The pH remained constant (7.74–7.75) throughout the study.

## MEASURED CONCENTRATIONS OF WSF OF SHALE JP-8

Results of weekly measurements of the WSF of shale JP-8 are presented in Table 15. During the egg-hatching period (Days 0–16) samples were withdrawn from the influent end of the tank. From Day 16–69 the fry resided in fry chambers and samples were withdrawn from these chambers. During the open-tank period following Day 69, samples were taken from the midpoint of the exposure tanks. No noticeable differences in WSF of shale JP-8 concentrations were observed that could be attributed to the sampling location or fuel age. The nominal 6% dilutions of fuel solubilizer products measured about half that amount (2.9% and 3.2%). Similarly, the nominal 13% dilutions measured approximately one-half (6.8% and 7.5%). The nominal 25% dilutions were reduced approximately one-third (to 18.2% and 17.9%). There was virtually no difference between the nominal 50% dilution and measured dilutions of 47.5% and 49.9%. The difference between nominal and measured dilutions is attributed primarily to biodegradation since shale JP-8 is not very volatile (see purging data in Materials and Methods).

## EGG HATCHING

Table 16 presents the results of egg hatching which show similar patterns in both banks of tanks. There was a pronounced acceleration of hatching rate as WSF of shale JP-8 concentration increased. This phenomenon was most evident on Days 11 and 12 of the 16-day hatching

TABLE 13

DILUTION WATER (GROUNDWATER) CHARACTERISTICS DURING FLAGFISH BIOASSAY

| Parameter | |
|---|---|
| Total solids, mg/ℓ | 527 ± 127 |
| Total dissolved solids, mg/ℓ | 540 ± 106 |
| Total hardness, mg as $CaCO_3$/ℓ | 318 ± 31 |
| $Cl^-$, mg/ℓ | 76.3 ± 5.3 |
| $SO_4^=$, mg/ℓ | 15.5 ± 4.0 |
| Conductivity, μmhos/cm | 832 ± 180 |
| pH | 7.7 ± 0.2 |

TABLE 14

EXPERIMENTAL CONDITIONS DURING FLAGFISH BIOASSAY

| Tank No. | Temperature, °C Mean ± s | DO, mg/ℓ Mean ± s | pH Mean ± s |
|---|---|---|---|
| Bank A | | | |
| 1 | 22.3 ± 0.7 | 8.4 ± 0.9 | 7.75 ± 0.06 |
| 2 | 22.3 ± 0.7 | 8.3 ± 0.8 | 7.75 ± 0.07 |
| 3 | 22.3 ± 0.7 | 8.2 ± 0.8 | 7.75 ± 0.07 |
| 4 | 22.3 ± 0.7 | 7.8 ± 0.7 | 7.75 ± 0.08 |
| 5 | 22.2 ± 0.7 | 7.6 ± 0.9 | 7.75 ± 0.08 |
| Bank B | | | |
| 1 | 22.2 ± 0.7 | 8.4 ± 0.9 | 7.75 ± 0.08 |
| 2 | 22.2 ± 0.7 | 8.4 ± 0.8 | 7.75 ± 0.08 |
| 3 | 22.3 ± 0.7 | 8.4 ± 0.8 | 7.75 ± 0.08 |
| 4 | 22.2 ± 0.7 | 8.0 ± 0.9 | 7.74 ± 0.09 |
| 5 | 22.2 ± 0.7 | 7.6 ± 0.9 | 7.74 ± 0.09 |

TABLE 15

WSF OF SHALE JP-8 CONCENTRATIONS DURING PARTIAL CHRONIC FLAGFISH BIOASSAY

mg/ℓ

| Expt. Day | Fuel Age days | 100% WSF of Shale JP-8 | Bank A Tanks | | | | Bank B Tanks | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 6% | 13% | 25% | 50% | 6% | 13% | 25% | 50% |
| 0 | 1 | 2.69, 2.90 | 0.11 | - | 0.38 | 1.48 | 0.07 | 0.24 | 0.37 | 1.36 |
| 3 | 3 | 2.33, 1.93 | 0.05 | 0.19 | 0.56 | 1.11 | 0.08 | 0.14 | 0.59 | 1.06 |
| 6 | 7 | 2.22, 2.44 | 0.06 | 0.19 | 0.31 | 0.95 | 0.09 | 0.19 | 0.25 | 1.08 |
| 11 | 5 | 2.94, 2.90 | 0.09 | 0.18 | 0.69 | 1.21 | 0.09 | 0.19 | 0.73 | 1.14 |
| 16 | 5 | 2.95, 2.66 | 0.12 | 0.28 | 0.78 | 1.53 | 0.09 | 0.22 | 0.43 | 1.30 |
| 24 | 3 | 3.02, 2.88 | 0.13 | 0.13 | 1.10 | 2.03 | 0.11 | 0.31 | 1.01 | 1.92 |
| 30 | 2 | 2.89, 2.64 | 0.16 | 0.30 | 0.90 | 1.90 | 0.15 | 0.28 | 1.01 | 1.81 |
| 38 | 3 | 2.94, 2.74 | 0.05 | 0.11 | 0.31 | 1.36 | 0.11 | 0.27 | 0.43 | 1.47 |
| 55 | 6 | 2.85, 3.11 | 0.12 | 0.24 | 0.99 | 1.83 | 0.12 | 0.32 | 1.10 | 1.92 |
| 62 | 6 | 2.54, 2.50 | 0.06 | 0.12 | 0.22 | 1.00 | 0.05 | 0.13 | 0.20 | 0.82 |
| 69 | 6 | 3.29, 3.24 | 0.04 | 0.22 | 0.33 | 1.45 | 0.05 | 0.11 | 0.37 | 1.91 |
| 76 | 6 | 3.21 | 0.04 | 0.12 | 0.16 | 1.32 | 0.03 | 0.26 | 0.48 | 1.24 |
| 81 | 4 | 2.40 | 0.04 | 0.10 | 0.15 | 1.06 | 0.05 | 0.10 | 0.15 | - |
| 97 | 6 | - | 0.11 | 0.23 | 0.63 | 1.55 | 0.13 | 0.30 | 0.70 | 1.86 |
| 101 | 3 | 2.92, 3.09 | 0.16 | 0.38 | 0.62 | 1.19 | 0.11 | 0.38 | 0.63 | 1.32 |
| 111 | 6 | 3.46, 3.33 | 0.01 | 0.09 | 0.17 | 1.13 | - | 0.04 | 0.13 | 1.05 |
| 118 | 6 | 2.50, 2.33 | 0.05 | 0.11 | 0.44 | 1.08 | 0.01 | 0.05 | 0.14 | 0.91 |
| 122 | 3 | 2.74, 3.03 | 0.09 | 0.26 | 0.37 | 0.77 | 0.11 | 0.19 | 0.33 | 1.04 |
| Mean: | | 2.80 | 0.08 | 0.19 | 0.51 | 1.33 | 0.09 | 0.21 | 0.50 | 1.37 |
| ± s: | | ±0.35 | ±0.04 | ±0.08 | ±0.29 | ±0.34 | ±0.04 | ±0.10 | ±0.31 | ±0.38 |
| % WSF: | | 100 | 2.9 | 6.8 | 18.2 | 47.5 | 3.2 | 7.5 | 17.9 | 49.9 |
| % Loss of WSF: | | | 3.1 | 6.2 | 6.8 | 2.5 | 2.8 | 5.5 | 7.1 | 0.1 |

TABLE 16

CUMULATIVE NUMBER OF FLAGFISH FRY HATCHED IN THE PRESENCE OF WSF OF SHALE JP-8

| Tank No. | WSF of Shale JP-8 Concn. mg/ℓ | Cumulative Number Hatched on Day: 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | Total Hatched % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bank A | | | | | | | | | | | |
| 1 | 0 | 0 | 0 | 0 | 3 | 19 | 123 | 128 | 138 | 142 | 86 |
| 2 | 0.08 | 0 | 0 | 0 | 6 | 30 | 133 | 134 | 138 | 141 | 85 |
| 3 | 0.19 | 0 | 0 | 0 | 2 | 50 | 147 | 147 | 147 | 147 | 89 |
| 4 | 0.51 | 0 | 0 | 0 | 10 | 98 | 156 | 156 | 156 | 156 | 94 |
| 5 | 1.3 | 0 | 16 | 39 | 106 | 129 | 141 | 141 | 141 | 141 | 85 |
| Bank B | | | | | | | | | | | |
| 1 | 0 | 0 | 0 | 0 | 2 | 5 | 75 | 112 | 129 | 131 | 80 |
| 2 | 0.09 | 0 | 0 | 0 | 2 | 8 | 124 | 126 | 134 | 136 | 82 |
| 3 | 0.21 | 0 | 0 | 0 | 5 | 30 | 130 | 131 | 135 | 137 | 83 |
| 4 | 0.50 | 0 | 0 | 0 | 19 | 48 | 139 | 141 | 141 | 141 | 85 |
| 5 | 1.4 | 0 | 0 | 12 | 36 | 64 | 117 | 117 | 117 | 117 | 70 |

period. The eventual hatching success was in the range of 70–94%, and was unaffected by any of the WSF of shale JP-8 concentrations tested.

The eggs hatched between Days 9 and 16. The acceleration of hatching was observed at all levels of fuel except the lowest (0.08 mg WSF of shale JP-8/ℓ). There were not a sufficient number of eggs hatched by Day 12 in the 0.08 mg/ℓ concentration to draw a conclusion with regard to acceleration of egg hatching, but there appears to be no effect.

The period of hatching was unusually long compared with the hatching period of previous studies (November 1979 Fourth Annual Report AMRL-TR-79-70). This was attributed to the low temperature ($19 \pm 0.9^{\circ}C$) that prevailed for the first 16 days of the experiment.

## SURVIVAL

Fish survival data are tabulated in Table 17. Thinning was performed on Days 24, 46, and 97. A graphic representation of survival (Figure 7) indicates that fuel had an effect at only the highest concentration, 1.35 mg WSF of shale JP-8/ℓ ($\chi^2_{calculated} = 55.34$, $\chi^2_{(0.95),\,4\,df} = 9.49$). There was no effect on survival at lower concentrations ($\chi^2_{calculated} = 1.40$, $\chi^2_{(0.95),\,3\,df} = 7.81$).

## GROWTH RATE

Fry growth rate was assessed by periodic length measurements. Measurements were obtained by a photographic method (described in June 1976 Annual Report AMRL-TR-76-50) on Days 24, 46, 60, and 74, and directly on Days 97 and 148. Photographic methods yielded total lengths rather than standard lengths. The results (depicted in Figure 8 and tabulated in Appendix 2) indicate that there was no apparent effect of the WSF of shale JP-8 concentrations tested on total fish length for 97 days. By Day 97 mortality had reduced the flagfish population exposed to the 1.35 mg WSF of JP-8/ℓ concentration below the planned thinning level. Since no fish were thinned, no length measurements could be performed on that day. During the period between Day 97 and Day 148 there was an apparent decrease in rate of growth of the flagfish exposed to the highest fuel concentration (1.35 mg WSF of shale JP-8/ℓ). This decrease was significant at the 1% level compared with the lengths of the flagfish in the other 4 exposure tanks. There was no significant difference at the 5% level in lengths of the fish in the 4 exposure tanks containing WSF of shale JP-8 concentrations of 0, 0.08, 0.20, and 0.51 mg/ℓ. The conclusion is that the no-effect level of WSF of shale JP-8 on flagfish growth as assessed by total length is between 0.51 and 1.35 mg/ℓ.

No significant difference (0.5%) in length was noted between banks of Tanks A and B.

## REPRODUCTION

On Day 97 the number of fish in each tank was selectively thinned (smaller fish removed) to approximately 30 (for the exact number and sex ratio see Table 18). These fish constituted the first generation, $F_1$, reared in the WSF of shale JP-8. At this time two spawning substrates were placed into each tank.

TABLE 17

SURVIVAL AND THINNING OF FLAGFISH EXPOSED TO WSF OF SHALE JP-8

| Tank Bank | WSF of Shale JP-8 Concn. mg/ℓ | Number of Eggs Added Day 0 | Number of Eggs Hatched Day 16 | Number of Survivors → Thinned to Number Specified | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Day 24 | Day 46 | Day 60 | Day 74 | Day 97 | Day 148 |
| A | 0 | 166 | 147 | 128 → 100 | 66 → 60 | 56 | 51 | 51 → 30 | 30 |
| | 0.08 | 166 | 141 | 128 → 100 | 71 → 60 | 60 | 57 | 55 → 30 | 30 |
| | 0.19 | 166 | 147 | 113 → 100 | 68 → 60 | 58 | 57 | 56 → 33 | 32 |
| | 0.51 | 166 | 156 | 123 → 100 | 74 → 60 | 59 | 54 | 52 → 30 | 29 |
| | 1.3 | 166 | 141 | 101 → 100 | 16 → 16 | 16 | 16 | 15 → 15 | 15 |
| B | 0 | 166 | 131 | 116 → 100 | 67 → 60 | 54 | 54 | 54 → 31 | 31 |
| | 0.09 | 166 | 136 | 121 → 100 | 70 → 60 | 59 | 55 | 54 → 30 | 29 |
| | 0.21 | 166 | 137 | 136 → 100 | 71 → 60 | 56 | 54 | 53 → 31 | 31 |
| | 0.50 | 166 | 141 | 130 → 100 | 75 → 60 | 54 | 45 | 41 → 30 | 29 |
| | 1.4 | 166 | 117 | 68 → 68 | 17 → 17 | 16 | 16 | 14 → 14 | 14 |

After 13 days no mating behavior had been observed — evidence that the males were still immature. Because time constraints did not permit a longer maturation period, a decision was made to add 2 older males that had not been reared in WSF of shale JP-8, an uncle backcross, to each tank. The immature males were left in the tank, but did not mate. Two days later mating behavior was observed by the older males and 23 days later on Day 133 the females deposited eggs.

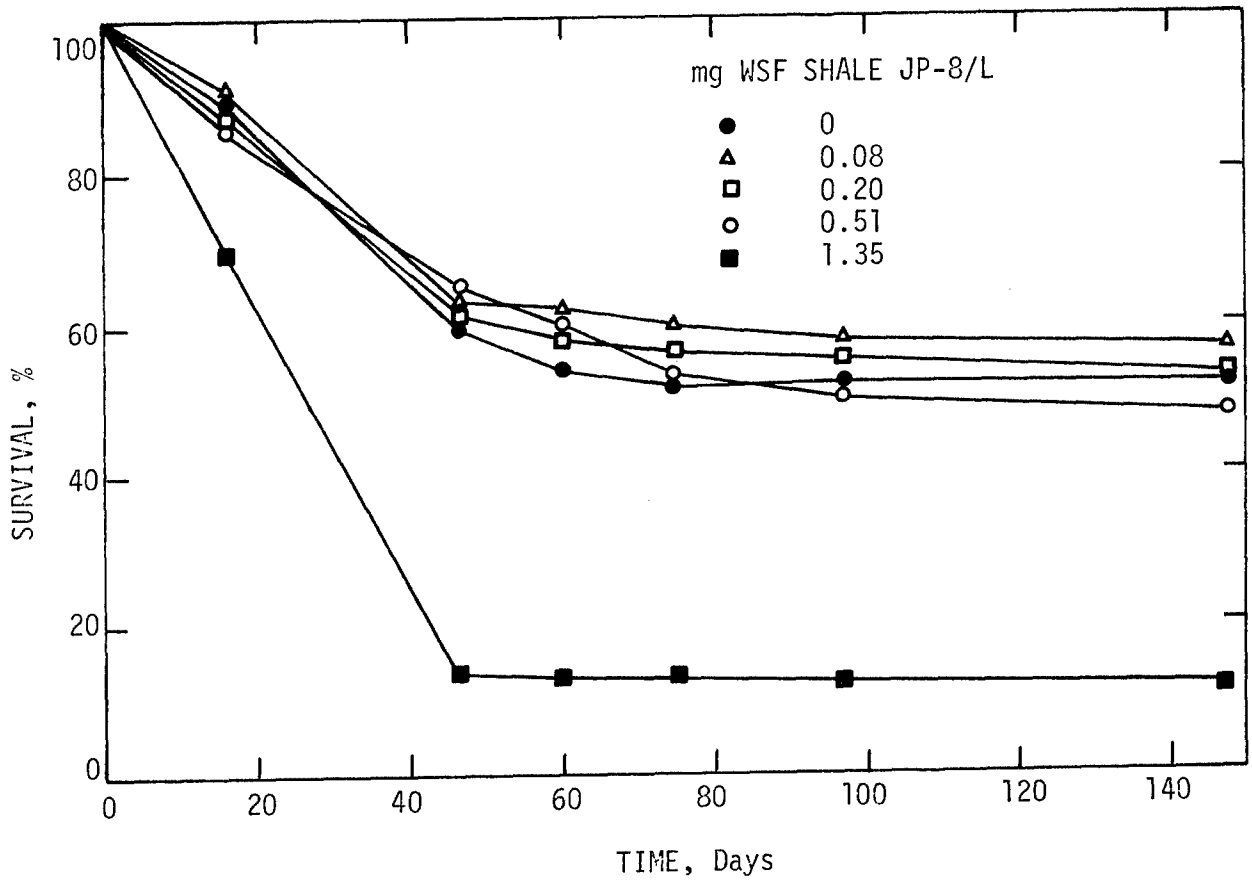



FIGURE 7. EFFECT OF WSF OF SHALE JP-8 ON FLAGFISH SURVIVAL (A + B BANKS)

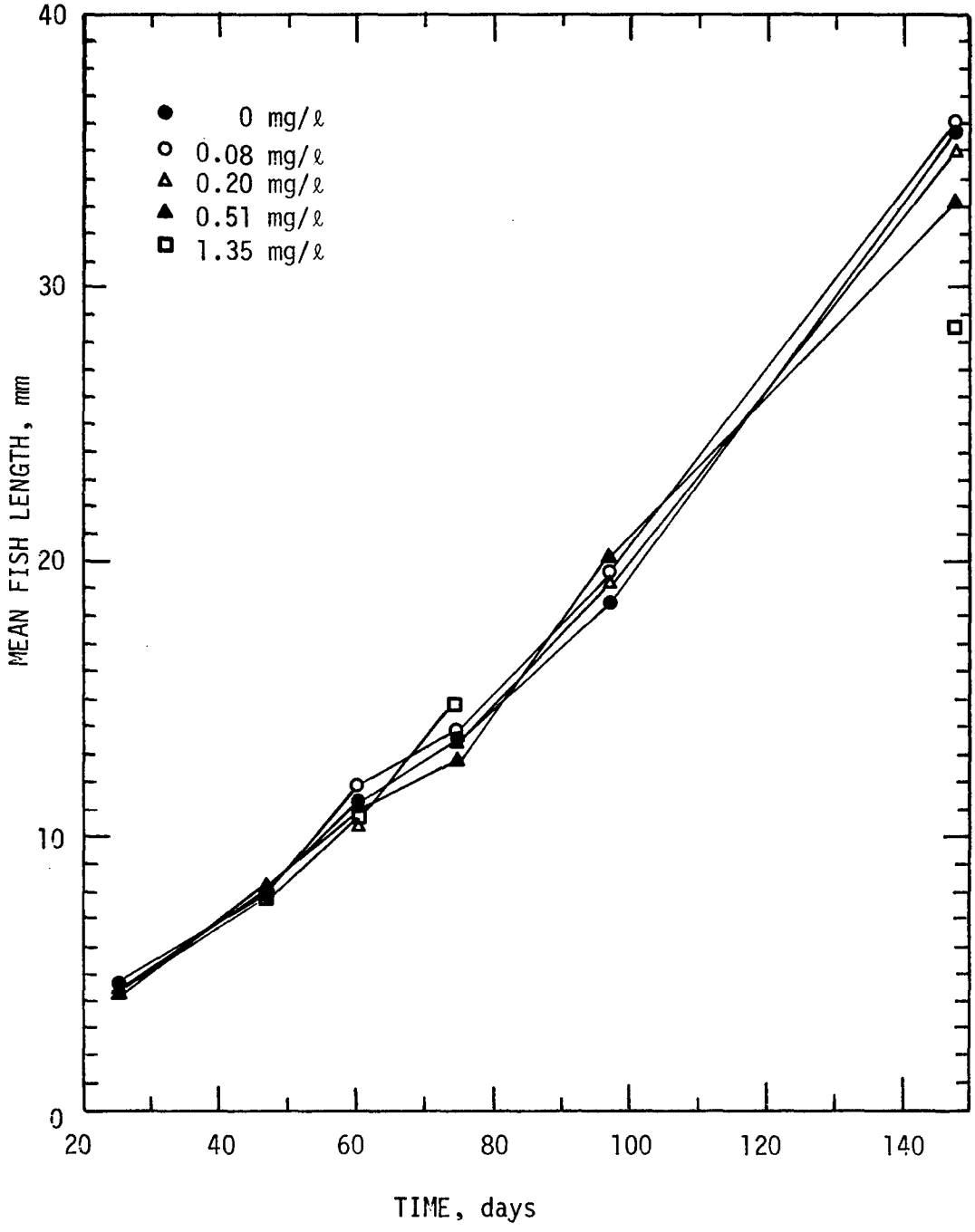



FIGURE 8. EFFECT OF WSF OF SHALE JP-8 ON FLAGFISH LENGTH

## TABLE 18

NUMBER OF $F_2$ FLAGFISH EGGS LAID ON GIVEN DAYS OF WSF OF SHALE JP-8 BIOASSAY

| Concn. of WSF of Shale JP-8 mg/ℓ | Adult Population | | Number of Eggs Laid on Day: | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Males No. | Females No. | 133 | 134 | 135 | 136 | 137 | 138 | 139 | 140 | 141 | 142 | 143 | 144 | 145 | 146 | 147 | 148 |
| Bank A | | | | | | | | | | | | | | | | | | |
| 0 | 8 | 22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 28 | 62 | 13 |
| 0.09 | 14 | 16 | 0 | 3 | 31 | 90 | 64 | 16 | 0 | 23 | 0 | 6 | 29 | 42 | 32 | 5 | 0 | 2 |
| 0.21 | 14 | 18 | 20 | 9 | 16 | 9 | 23 | 0 | 2 | 4 | 0 | 0 | 29 | 26 | 0 | 0 | 0 | 2 |
| 0.54 | 13 | 16 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 21 | 38 |
| 1.26 | 3 | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Bank B | | | | | | | | | | | | | | | | | | |
| 0 | 23 | 8 | 0 | 0 | 0 | 4 | 29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.08 | 15 | 14 | 2 | 0 | 0 | 0 | 40 | 18 | 68 | 0 | 0 | 0 | 83 | 17 | 56 | 0 | 6 | 2 |
| 0.20 | 11 | 20 | 2 | 0 | 0 | 0 | 1 | 0 | 27 | 30 | 19 | 23 | 39 | 27 | 4 | 2 | 4 | 2 |
| 0.47 | 7 | 22 | 0 | 0 | 0 | 0 | 10 | 0 | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 12 |
| 1.19 | 3 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Substrates were examined daily for eggs. Deposited eggs were immediately counted, transferred to egg cups, treated with Malachite Green, and returned to their respective tanks. Agitation and aeration was provided by placing egg cups under the influent stream to the tank. As additional eggs were deposited on a day-by-day basis, the process was repeated and the eggs were added to the egg cups. Degenerated eggs were removed from the egg cups on a daily basis. Fresh substrates were placed in the tanks daily to replace substrates that had received eggs.

On Day 133 the fish in 0.20 mg WSF of shale JP-8/ℓ began depositing eggs. In the next two days there were considerable numbers of eggs deposited in both 0.09 and 0.20 mg WSF of shale JP-8/ℓ. In the control there were no eggs deposited until Day 145. The presence of low concentrations of WSF of shale JP-8, therefore, appeared to accelerate deposition of eggs. There were fewer total eggs deposited in the control, but the experiment had to be curtailed.

There were no eggs laid at the highest fuel concentration (1.19—1.26 mg WSF of shale JP-8/ℓ) and at the termination of the experiment on Day 148 the fish at this WSF of shale JP-8 concentration had not displayed mating behavior.

Table 19 summarizes the total number of eggs laid and the number of $F_2$ fry hatched. The variability in the controls tends to negate any conclusion.

In the duplicate tanks of 0.51 mg WSF of shale JP-8/ℓ concentration, some of the fry hatched only partially out of their egg cases. By the following day these fry were completely hatched and appeared normal.

## SALINE WATER BIOASSAYS OF HYDROCARBON FUELS

### INTRODUCTION

The effect of hydrocarbon fuels on estuarine biota was examined in a series of bioassays designed to determine the LC 50 and no-effect level of WSF's of shale JP-8, JP-8, and JP-4 on stickleback and aufwuchs. Acute 96-hr range-finding bioassays were followed by chronic 2- or 3-week continuous-flow bioassays. Preliminary work demonstrated that it was necessary to conduct separate bioassays on aufwuchs and stickleback because interactions between the two caused uncontrollable DO fluctuations in the small-scale vessels utilized for these bioassays. At the lower fuel concentrations there was more abundant algal growth which coated the fish cages and interfered with water circulation despite daily maintenance. Separating the study of fish and aufwuchs meant that the fish cages could be discarded, since there was no longer a need to confine the fish. With the fish swimming freely in the tanks, there were no further DO problems.

TABLE 19

$F_2$ EGG HATCHING OF FLAGFISH EXPOSED TO WSF OF SHALE JP-8

| Concn. of WSF of Shale JP-8 mg/ℓ | Eggs Laid No. | Eggs Discarded No. | $F_2$ Fry Hatched No. | Viable Eggs % | Degener-ated Eggs No. | Un-accounted Eggs No. |
|---|---|---|---|---|---|---|
| Bank A | | | | | | |
| 0 | 103 | 40* | 50 | 87 | 6 | 7 |
| 0.09 | 337 | 10 | 144† | 43† | 7 | 176† |
| 0.21 | 140 | 6 | 118 | 84 | 14 | 2 |
| 0.54 | 61 | 32* | 23 | 90 | 0 | 6 |
| 1.26 | 0 | 0 | 0 | 0 | 0 | 0 |
| Bank B | | | | | | |
| 0 | 33 | 0 | 2 | 6 | 27 | 4 |
| 0.08 | 292 | 10 | 179† | 61† | 13 | 90† |
| 0.20 | 180 | 15 | 133 | 74 | 6 | 26 |
| 0.47 | 59 | 0 | 48 | 81 | 8 | 3 |
| 1.19 | 0 | 0 | 0 | 0 | 0 | 0 |
| Banks A + B | | | | | | |
| 0 | 136 | 40* | 52 | 68 | 33 | 11 |
| 0.09 | 626 | 20 | 323† | 52† | 20 | 283† |
| 0.21 | 320 | 21 | 251 | 78 | 20 | 28 |
| 0.51 | 120 | 32* | 71 | 86 | 8 | 9 |
| 1.22 | 0 | 0 | 0 | 0 | 0 | 0 |

* Discarded normal appearing eggs (time constraints required termination of experiment); these eggs included in % viability.

† An unknown number of eggs were lost accidentally.

## STATIC BIOASSAY — STICKLEBACK EXPOSED TO WSF OF JP-4

### Introduction

A 96-hr static acute test was performed to determine the LC 50 and estimate the toxicant levels for use in a long-term continuous-flow bioassay. WSF of JP-4 was prepared by continuously mixing 5% JP-4 in Bay water for 18 hr, then by settling for 4 to 6 hr, then withdrawing the WSF.

### Procedure

The following duplicate volumetric concentrations of the WSF were prepared daily: 0% (control), 32%, 56%, 65%, 75%, and 87%. GC measurement of concentrations (mg/ℓ) of WSF of JP-4 were performed using the purge-and-trap method.

Stickleback were acclimated in Bay water (salinity = 26.5 ± 1.9 °/∞ ; temperature = 18.5 ± 1.9°C) for 21 days prior to the experiment. Ten stickleback each were exposed to 15 ℓ of test solution contained in 19.5-ℓ glass jars covered with aluminum foil.

### Results

The initial 100% WSF of JP-4 concentration was 12.5 mg/ℓ based on GC measurement. The concentration of each dilution was estimated from this value because air bubbles developed in sample storage bottles and would have negated GC analyses on these dilutions. In subsequent work, sample storage bottles were sealed with rubber septa to prevent air bubble formation.

Experimental parameters are presented in Table 20 and results of stickleback mortality are presented in Table 21. At the two highest concentrations (87 and 75% WSF of JP-4) mean mortalities were 95 and 90%, respectively; no deaths were observed in the remaining WSF concentrations (0 to 65%). Despite the absence of deaths at these concentrations, fish discoloration occurred at the 56 and 65% WSF of JP-4 concentrations. In addition, some of the fish exposed to the higher WSF of JP-4 concentrations exhibited a reddish-pink hemorrhaging and swelling under the mouth. Several of these fish died within a few days following the 96-hr period, despite being removed from the presence of fuel.

The nominal 96-hr LC 50 was estimated at approximately 72% WSF of JP-4 (9.0 mg/ℓ) based on initially added concentrations. The actual WSF of JP-4 concentration would be expected to be substantially lower than this value because of volatility and/or biodegradation losses following each daily fuel renewal. Volatility studies are reported in Appendix 3.

TABLE 20

EXPERIMENTAL CONDITIONS DURING 96-hr WSF OF JP-4 STATIC BIOASSAY

| Parameter | Value |
|---|---|
| Salinity, °/∞ | 26.2 ± 1.1 |
| Temperature, °C | 20.3 ± 3.5 |
| pH | 8.4 ± 0.2 |
| DO, mg/ℓ | 8.5 ± 0.7 |
| Fish length, mm | 39.0 ± 0.9 |

## CONTINUOUS BIOASSAY - STICKLEBACK EXPOSED TO WSF OF JP-4

### Procedure

Twenty fish were exposed to each of the following duplicate volumetric concentrations of WSF of JP-4: 0%, 10%, 40%, 70%, and 100%. The mean length and wet weight of the fish were 37.4 ± 0.8 mm and 0.41 ± 0.02 g, respectively.

WSF of JP-4 concentrations were analyzed by the purge-and-trap GC method three times during the 168-hr experiment at periods of 20, 40, and 68 hr following fuel replenishment. Salinity, temperature, pH, and DO were measured daily and alkalinity, chloride, and total dissolved solids were analyzed at 0 and 168 hr.

### Results

Results of the Bay water analyses are compiled in Table 22. The water temperature (12°C) was much colder than that recorded in the previous stickleback experiment (20.3°C).

TABLE 21

STICKLEBACK MORTALITY IN 96-hr WSF OF JP-4 STATIC BIOASSAY

| Est. WSF of JP-4 Conc. | | Cumulative Stickleback Mortality After Hour: | | | | |
|---|---|---|---|---|---|---|
| Volume, % | mg/ℓ | 24 | 48 | 72 | 96 | %Mortality |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 32 | 4.0 | 0 | 0 | 0 | 0 | 0 |
| 32 | 4.0 | 0 | 0 | 0 | 0 | 0 |
| 56 | 7.0 | 0 | 0 | 0 | 0 | 0 |
| 56 | 7.0 | 0 | 0 | 0 | 0 | 0 |
| 65 | 8.1 | 0 | 0 | 0 | 0 | 0 |
| 65 | 8.1 | 0 | 0 | 0 | 0 | 0 |
| 75 | 9.4 | 0 | 1 | 1 | 9 | 90 |
| 75 | 9.4 | 0 | 0 | 0 | 9 | 90 |
| 87 | 10.9 | 1 | 7 | 7 | 9 | 90 |
| 87 | 10.9 | 0 | 10 | 10 | 10 | 100 |

TABLE 22

EXPERIMENTAL CONDITIONS FOR CONTINUOUS-FLOW BIOASSAY OF WSF OF JP-4 WITH STICKLEBACK

| Parameter | Value |
|---|---|
| Salinity, $^{o}/_{oo}$ | 28.6 ± 3.1 |
| Temperature, $^{o}C$ | 12.2 ± 2.6 |
| pH | 7.83 ± 0.07 |
| DO, mg/ℓ | 7.6 ± 0.7 |
| Alkalinity, mg as $CaCO_3$/ℓ | 131 ± 17 |
| Chloride, g/ℓ | 10.9 ± 4.9 |
| TDS, g/ℓ | 28.7 ± 4.1 |

The WSF of JP-4 analyses (Table 23 ) had a high relative standard deviation (RSD) for the lowest WSF of JP-4 level (10% dilution) in both banks of tanks, and for the 40% dilution of WSF of JP-4 in the Bank A tank. The other WSF of JP-4 analyses showed reasonable consistency.

Although duplication of bioassay results between banks of tanks was far from perfect, there was a satisfactory mean concentration gradient in each bank of tanks. In Bank A the measured WSF of JP-4 concentrations in the test tanks were about 3, 8, and 10% higher than in the nominal concentrations of 10, 40, and 70%, respectively. The 100% exposure tank mean concentration was 9% lower than the 100% solubilizer product (measured in the manifold).

Stickleback mortality (Table 24 ) indicates a positive correlation with increasing WSF of JP-4 concentration. No deaths occurred in the controls or in the tanks containing < 1.4 mg WSF of JP-4/ℓ.

There was significant mortality at the higher WSF of JP-4 concentrations: at concentrations of 5.1, 6.5, and 8.5 mg/ℓ mortalities of 20, 45, and 75%, respectively, were observed after 168 hr. At concentrations of ≥ 8.9 mg/ℓ, 100% mortality occurred. The 168-hr LC 50 was 6.80 mg/ℓ with 95% confidence limits of 6.14 to 7.53 mg/ℓ.

## Discussion

The study lasted for only 7 days instead of the planned 14-day period because of a mechanical failure of the Bay water filtering pump.

The 168-hr LC 50 of 6.8 mg WSF of JP-4/ℓ appears to be consistent with the previously-obtained 96-hr LC 50 of 9.0 mg WSF of JP-4/ℓ.

In the present study fish mortality was observed at WSF of JP-4 concentrations > 5 mg/ℓ. Those fish that survived at > 5 mg WSF of JP-4/ℓ became dark colored and exhibited a stressed behavior characterized by abnormal swimming patterns and respiratory difficulty. A majority of the dead fish had been hemorrhaging and had enlarged gill operculum and contraction of the dorsal and pectoral spines.

## CONTINUOUS BIOASSAY – AUFWUCHS EXPOSED TO WSF OF JP-4

## Procedure

An aufwuchs rack was suspended 18 cm below the water surface of each of the 10 test tanks. After 504 hr (3 weeks) 6 substrates from each rack were removed and measurements of respiration, biomass, and chlorophyll content were performed. The seventh substrate from each rack was preserved for future identification of individual species.

The aufwuchs were grown in the following duplicate volumetric dilutions of solubilizer product: 0% (control), 20%, 40%, 70%, and 100%. Routine maintenance included daily cleaning to remove extraneous algal growths and daily calibration of flow rates.

TABLE 23

WSF OF JP-4 CONCENTRATIONS IN BIOASSAY EXPOSURE TANKS

| Tank Bank | Experimental Time hr | Fuel Age hr | WSF of JP-4 Concn., mg/ℓ at Indicated % Dilution of Solubilizer Product | | | | | 100% (Fuel Solubilizer Product) |
|---|---|---|---|---|---|---|---|---|
| | | | 0% | 10% | 40% | 70% | 100% | |
| A | 0 | 20 | 0 | 1.32 | 7.17 | 8.95 | 10.64 | 11.76 |
| | 120 | 68 | 0 | 1.04 | 3.34 | 7.72 | 8.51 | 9.72 |
| | 168 | 40 | 0 | 1.81 | 4.70 | 8.70 | -- | 10.14 |
| | Mean: | | 0 | 1.39 | 5.07 | 8.46 | 9.58 | 10.54 |
| | s: | | | ±0.39 | ±1.94 | ±0.65 | ±1.51 | |
| | RSD, %: | | | 28.1 | 38.3 | 7.7 | 15.8 | |
| | * WSF, %: | | | 13.2 | 48.1 | 80.3 | 90.9 | |
| B | 0 | 20 | 0 | 0.32 | 6.38 | 8.32 | 10.81 | |
| | 120 | 68 | 0 | 0.94 | 6.67 | 9.49 | 12.04 | |
| | 168 | 40 | 0 | 1.94 | -- | -- | -- | |
| | Mean: | | 0 | 1.07 | 6.53 | 8.91 | 11.43 | |
| | s: | | | 0.82 | 0.21 | 0.83 | 0.87 | |
| | RSD, %: | | | 76.6 | 3.2 | 9.3 | 7.6 | |
| | * WSF %: | | | 10.2 | 62.0 | 84.5 | 108.4 | |

* Based on a solubilizer product concentration of 10.54 mg/ℓ being 100%.

The fuel solubilizers were cleaned and replenished with fuel at 68-hr intervals. Measurement of JP-4 concentrations in the test tanks was performed 7 times (twice at fuel ages of 20, 44, and 68 hr and once at a fuel age of 92 hr. Salinity, temperature, pH, and DO were measured daily and alkalinity, chloride, and TDS were analyzed weekly.

## Results

Bay water characteristics, presented in Table 25, indicate some fluctuation in alkalinity and temperature. The latter was because of the overcast condition that prevailed during the first 7 days of the study. The absence of a steady photoperiod hindered aufwuchs development and therefore the experiment was extended. The final 14 days of the experiment provided an excellent photoperiod despite two heavy rainstorms.

TABLE 24

TOXICITY OF WSF OF JP-4 TO STICKLEBACK

| Nominal WSF of JP-4 Concn., % | WSF of JP-4 Concn., mg/ℓ | Number of Test Organisms | Cumulative No. of Test Organisms Dead After Hour: 24 | 48 | 72 | 96 | 120 | 144 | 168 | Mortality, % |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 1.39 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | 1.07 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 40 | 5.07 | 20 | 0 | 2 | 4 | 4 | 4 | 4 | 4 | 20 |
| 40 | 6.53 | 20 | 0 | 1 | 2 | 2 | 2 | 4 | 9 | 45 |
| 70 | 8.46 | 20 | 0 | 7 | 7 | 8 | 8 | 8 | 15 | 75 |
| 70 | 8.91 | 20 | 0 | 6 | 8 | 11 | 19 | 19 | 20 | 100 |
| 100 | 9.58 | 20 | 14 | 20 | 20 | 20 | 20 | 20 | 20 | 100 |
| 100 | 11.43 | 20 | 14 | 18 | 20 | 20 | 20 | 20 | 20 | 100 |

Results of GC analysis of WSF of JP-4 are compiled in Table 26. The WSF of JP-4 concentrations in banks of tanks A and B exhibited a regular mass concentration gradient with % dilution. The duplication between the two banks of tanks was excellent. While past experience had shown that measured concentrations were usually lower than would be predicted from the % dilutions of solubilizer product because of volatility and/or biodegradation losses, the measured concentrations in this experiment averaged 15, 20, and 4% higher than the selected nominal concentrations of 20, 40, and 70%, respectively. This is attributable to flow rate variation and analytical error. The 100% concentrations were slightly lower than those measured in the solubilizer manifolds because of volatilization from the exposed tank surface. A decline in fuel concentration as a function of fuel age was apparent for the 92-hr samples.

TABLE 25

EXPERIMENTAL CONDITIONS DURING CONTINUOUS-FLOW BIOASSAY OF WSF OF JP-4 WITH AUFWUCHS

| Parameter | Value |
|---|---|
| Salinity, °/oo | 26.9 ± 6.3 |
| Temperature, °C | 15.0 ± 2.7 |
| pH | 7.8 ± 0.2 |
| DO, mg/ℓ | 7.2 ± 1.2 |
| Alkalinity, mg as $CaCO_3$/ℓ | 116.8 ± 2.1 |
| Chloride, g/ℓ | 16.0 ± 0.5 |
| TDS, g/ℓ | 34.5 ± 2.2 |

The results of biomass, chlorophyll, and productivity measurements are tabulated in Table 27. One-way analysis of variance performed on the dry weight, organic content, chlorophyll, and gross photosynthetic data indicated highly significant ($p < 0.001$) differences in each of these parameters as a function of fuel concentration.

The dry weights and organic contents of aufwuchs biomass consistently decreased from control values with increasing WSF of JP-4 concentration. The control aufwuchs had a biomass 4–8 times greater than the aufwuchs exposed to the various WSF of JP-4 concentrations. The organic content of the control aufwuchs biomass was approximately 14, 22, and 36% greater than for aufwuchs exposed to approximately 3 mg/ℓ, 6–7 mg/ℓ, and 9 mg/ℓ WSF of JP-4.

Chlorophyll concentrations also showed similar trends with respect to increasing WSF of JP-4 concentration. Chlorophyll a and c were the most sensitive indicators of toxicity. Neither chlorophyll b nor pheophytin a appeared to be definitive toxicity indicators.

Gross photosynthesis clearly showed a response to toxicant which reflected the diminished aufwuchs growth at increasing fuel concentrations. The photosynthetic index with respect to weight ($PI_{wt}$) was sensitive to increasing toxicant concentrations indicating that the metabolic processes were affected. Photosynthetic index with respect to chlorophyll ($PI_{chl\ a}$) increased with increasing WSF of JP-4 concentrations, excluding the two highest exposures.

TABLE 26

WSF OF JP-4 CONCENTRATIONS, mg/ℓ

| Tank Bank | Experimental Time hr | Fuel Age hr | Mass Concentrations of WSF of JP-4 at Indicated % Dilutions of Solubilizer Product | | | | | 100% (Fuel Solubilizer Product) |
|---|---|---|---|---|---|---|---|---|
| | | | 0% | 20% | 40% | 70% | 100% | |
| A | 0 | 20 | 0 | 3.4 | 5.3 | 8.4 | 9.4 | -- |
| | 144 | 44 | 0 | 4.1 | 5.6 | 8.9 | 9.9 | 10.4 |
| | 288 | 20 | 0 | 1.8 | 7.1 | 5.3 | 8.1 | 11.6 |
| | 336 | 68 | 0 | 3.5 | 5.8 | 6.9 | 11.0 | 11.9 |
| | 384 | 44 | 0 | 3.5 | 7.6 | 8.3 | 9.0 | 8.8 |
| | 504 | 92 | 0 | 3.2 | 5.2 | 6.9 | 7.6 | 8.2 |
| | Mean: | | 0 | 3.3 | 6.1 | 7.5 | 9.2 | 10.2 |
| | s: | | | ±0.78 | ±1.03 | ±1.37 | ±1.23 | ±1.64 |
| | RSD, %: | | | 24.0 | 16.9 | 18.4 | 13.4 | 16.1 |
| | WSF, %: | | | 31.9 | 59.7 | 73.1 | 89.8 | |
| B | 0 | 20 | 0 | 4.1 | 6.5 | 8.6 | 10.7 | -- |
| | 144 | 44 | 0 | 4.1 | 6.0 | 8.0 | 10.0 | 10.5 |
| | 288 | 20 | 0 | 4.1 | 7.1 | 4.6 | 10.1 | 10.5 |
| | 336 | 68 | 0 | 3.3 | 5.5 | 7.7 | 9.8 | 10.4 |
| | 384 | 44 | 0 | 3.1 | 4.4 | 8.2 | 9.6 | 9.8 |
| | 504 | 92 | 0 | 2.5 | 4.6 | 6.1 | 7.2 | 7.8 |
| | Mean: | | 0 | 3.5 | 5.7 | 7.2 | 9.6 | 9.6 |
| | s: | | | ±0.67 | ±1.07 | ±1.53 | ±1.22 | ±1.26 |
| | RSF, %: | | | 19.0 | 18.9 | 21.3 | 12.7 | 13.1 |
| | WSF, %: | | | 36.7 | 58.9 | 74.8 | 99.4 | |

TABLE 27

STANDING CROP AND PRODUCTIVITY OF AUFWUCHS COMMUNITIES EXPOSED TO FIVE CONCENTRATIONS OF WSF OF JP-4 FOR 504 HOURS

| JP-4 Concn. mg/ℓ | Standing Crop: Biomass Dry Wt. mg/Auf. | Biomass %Organic | Chlorophyll a mg/g Dry Wt. | Chlorophyll b mg/g Dry Wt. | Chlorophyll c mg/g Dry Wt. | Pheophytin a mg/g Dry Wt. | Gross Photosynthesis mg $O_2$/Auf-hr | $PI_{(wt)}$ mg $O_2$ Dry wt-hr | $PI_{(Chl\ a)}$ mg $O_2$ Chl a-hr |
|---|---|---|---|---|---|---|---|---|---|
| 0.0 | 27.5 | 26 | 18 | 1.6 | 9.3 | 0.99 | 2.08 | 76 | 4 |
| 0.0 | 35.4 | 23 | 18 | 2.2 | 11 | 3.1 | 4.79 | 135 | 7 |
| 3.3 | 9.0 | 22 | 17 | 1.1 | 5.9 | 0 | 1.42 | 158 | 9 |
| 3.5 | 7.7 | 20 | 18 | 1.3 | 6.8 | 0 | 1.59 | 205 | 11 |
| 6.1 | 5.7 | 20 | 7.7 | 2.5 | 0.88 | 0.25 | 0.47 | 82 | 11 |
| 5.7 | 5.2 | 19 | 3.5 | 1.3 | 0.12 | 0.06 | 0.25 | 48 | 14 |
| 7.4 | 4.7 | 18 | 2.3 | 0.93 | 0.07 | 0.31 | 0.02 | 4 | 2 |
| 7.2 | 6.5 | 20 | 2.3 | 0.73 | 0.16 | 0.04 | 0.06 | 9 | 4 |
| 9.2 | 4.5 | 16 | 0.29 | 0.15 | 0 | 0.34 | -0.07 | -16 | -54 |
| 9.6 | 3.7 | 15 | 0.41 | 0.18 | 0 | 0.09 | 0.03 | 8 | 20 |

## Discussion

This experiment clearly demonstrated the toxic effect of the WSF of JP-4 on aufwuchs. Fuel concentrations greater than 3.0 mg/ℓ, and especially those greater than 5.0 mg/ℓ, drastically affected growth. Based on these results it is concluded that the no-effect level is less than 3.0 mg/ℓ (the lowest level tested).

Gross photosynthesis, total biomass, and chlorophyll c appeared to be the best indicators of WSF of JP-4 toxicity to aufwuchs.

# STATIC BIOASSAY - STICKLEBACK EXPOSED TO WSF OF SHALE JP-8

## Introduction

A static bioassay was performed to determine the LC 50 of the WSF of shale JP-8 to stickleback and to estimate the concentrations required for a continuous-flow bioassay.

## Procedure

The following volumetric dilutions of solubilizer product of JP-8 were selected: 0% (control), 18%, 32%, 64%, 87%, and 100%. Each concentration was prepared in duplicate and renewed every 24 hr.

Stickleback (mean length, 41.8 ± 1.2 mm; mean wet weight, 0.58 ± 0.07 g) were acclimated for 7 days prior to the start of the experiment. Ten fish each were exposed to 10 ℓ of toxicant contained in 5-gal (19.5 ℓ) glass jars covered with aluminum foil.

The initial and final WSF of shale JP-8 concentrations were measured by GC analysis during each of two 24-hr experimental periods (0–24 hr and 24–48 hr). These samples were taken to ascertain the decrease in fuel concentration before fuel renewal.

During acclimation, water salinity was 28.7 ± 2.5 °/oo and the temperature was 11.7 ± 1.5 °C. During the experiment salinity, temperature, pH, and DO were measured at 24-hr intervals. Fish mortality and behavior also were observed at these intervals.

## Results

The results of the Bay water and test solution water quality analyses are presented in Table 28.

Table 29 presents the results of GC analyses of the WSF of shale JP-8 concentrations at the initial preparation of the solution ( Δ0 ) and after 24 hr ( Δ24 ) for the two periods examined. The 100% WSF of shale JP-8 concentration was in the range 2.2–2.6 mg/ℓ. The measured initial WSF of shale JP-8 concentrations were in close agreement with the volumetric dilutions particularly at the outset of the second period. After 24 hr there was a considerable depletion in WSF of shale JP-8 concentration.

TABLE 28

EXPERIMENTAL CONDITIONS DURING STATIC WSF OF SHALE JP-8 BIOASSAY

| Parameter | Value |
|---|---|
| Salinity, °/oo | 28.3 ± 3.0 |
| Temperature, °C. | 12.0 ± 2.0 |
| pH | 7.76 ± 0.09 |
| DO, mg/ℓ | 8.3 ± 0.3 |
| Alkalinity, mg as $CaCO_3$/ℓ. | 128.0 ± 46.7 |
| Chloride, g/ℓ | 13.0 ± 1.9 |
| TDS, g/ℓ | 28.6 ± 2.6 |

The mean loss of WSF of shale JP-8 during each 24-hr interval was 30-40%.

There were no fish deaths in any of the test solutions during the 96-hr period. However, fish exposed to the two higher fuel concentration levels (87 and 100% initial dilutions of WSF of shale JP-8) exhibited severe stress behavior characterized by increased respiration rate, swimming at a 45 deg. angle to the surface, sluggish movement, and dark coloration. At the two intermediate WSF of shale JP-8 concentrations (32 and 65%) there were fewer fish stressed than at the higher concentrations. At the lowest fuel level (18% initial dilution of WSF of shale JP-8) there was no difference in fish behavior or appearance when compared with the control fish.

Based on these results, the concentrations in the continuous-flow 336-hr experiment were selected to be the same as in the static 96-hr bioassay.

## CONTINUOUS FLOW BIOASSAY - STICKLEBACK TO WSF OF SHALE JP-8

### Introduction

A 336-hr study of stickleback exposed to WSF of shale JP-8 was conducted to determine the no-effect level with respect to fish survival and the LC 50 concentration.

### Procedure

Stickleback (mean length 40.0 ± 0.7 mm; mean wet weight 0.46 ± 0.04 g) were acclimated for 7 days to Bay water. During the

TABLE 29

MEASURED WSF OF SHALE JP-8 CONCENTRATIONS AND LOSSES DURING 24-hr INTERVALS: 96-hr STATIC BIOASSAY WITH STICKLEBACK

| Nominal WSF of Shale JP-8 % | Measured Concentration of WSF of Shale JP-8 | | | | Mean* Measured % WSF of Shale JP-8 | | Mean** Loss |
|---|---|---|---|---|---|---|---|
| | Bank A | | Bank B | | | | |
| | Δ0 | Δ24 hr | Δ0 | Δ24 hr | Δ0 | Δ24 hr | Δ 0 - Δ24, % |
| | | | Period 0–24 hr | | | | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | - | - | 0.20 | 0.12 | 9 | 6 | 40 |
| 32 | 0.51 | 0.22 | 0.63 | 0.26 | 26 | 11 | 57 |
| 65 | - | - | 1.2 | 0.89 | 54 | 41 | 25 |
| 87 | 1.7 | 0.67 | 1.7 | 1.2 | 79 | 44 | 44 |
| 100 | 2.1 | 1.7 | 2.2 | 1.6 | 100 | 76 | 24 |
| | | | Period 24–48 hr | | | | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | 0.37 | 0.20 | 0.34 | 0.21 | 14 | 8 | 42 |
| 32 | 0.62 | 0.31 | 0.99 | 0.35 | 31 | 13 | 38 |
| 65 | 1.6 | 0.95 | 1.6 | 1.1 | 60 | 38 | 35 |
| 87 | 2.2 | 1.5 | 2.4 | 1.5 | 87 | 57 | 34 |
| 100 | 2.6 | 1.8 | 2.6 | 1.8 | 100 | 70 | 30 |

* Mean of Bank A and Bank B duplicates expressed as a percentage of the mean Δ0 100 value, i.e., 2.15.

** Mean of Bank A and Bank B duplicate loss during the 24-hr period.

acclimation period the salinity was 8.0 ± 3.3 °/oo and the temperature was 16.3 ± 1.8 °C.

Twenty fish each were exposed to the following duplicate volumetric concentrations of WSF of shale JP-8: 0% (control), 18%, 32%, 65%, and 100%. Flow rates were calibrated daily and the solubilizer was cleaned after 1 week.

The fuel concentrations in the exposure tanks were analyzed 4 times during the experiment – twice each at fuel ages of 20 and 168 hr. Daily measurements were taken of salinity, temperature, pH, and DO. Alkalinity, chloride, and TDS were analyzed at 0, 168, and 336 hr.

## Results

The Bay water parameters (Table 30 ) indicate lower than normal salinity, chloride, and TDS attributable to the dilution by unusually heavy fresh water inflow to San Francisco Bay.

TABLE 30

WATER QUALITY DURING CONTINUOUS-FLOW WSF OF SHALE JP-8 BIOASSAY WITH STICKLEBACK

| Parameter | Value |
|---|---|
| Salinity, °/oo | 10.2 ± 3.6 |
| Temperature, °C | 15.1 ± 3.6 |
| pH | 8.03 ± 0.2 |
| DO, mg/ℓ | 8.6 ± 0.8 |
| Alkalinity, mg as $CaCO_3$/ℓ | 110 ± 61 |
| Chloride, g/ℓ | 4.6 ± 1.0 |
| TDS, g/ℓ | 10.1 ± 2.2 |

The results of GC analyses of the WSF of shale JP-8, tabulated in Table 31 , show a regular concentration gradient in both banks of tanks. Comparison of the means of replicate WSF of JP-8 dilutions in the two banks of tanks indicates that duplication was excellent in the middle two concentrations (32 and 65% WSF of shale JP-8), but was poor in the lowest and highest exposures, 18 and 100% WSF of shale JP-8, respectively. Generally, the measured values were reasonably close to the selected % dilutions of WSF of shale JP-8.

TABLE 31

MEASURED WSF OF SHALE JP-8 CONCENTRATIONS, mg/ℓ

| Tank Bank | Experimental Time hr | Fuel Age hr | WSF of Shale JP-8 Concentrations (mg/ℓ) at Indicated % Dilutions of Solubilizer Product 0% | 18% | 32% | 65% | 100% | 100% (Fuel Solubilizer Product) |
|---|---|---|---|---|---|---|---|---|
| A | 0 | 20 | 0 | 0.21 | 0.94 | 1.7 | 2.7 | 2.6 |
| | 168 | 168 | 0 | 0.32 | 1.0 | 2.3 | 2.9 | 2.0 |
| | 192 | 20 | 0 | 0.22 | 0.92 | 1.8 | -- | 3.8 |
| | 336 | 168 | 0 | 0.22 | 1.0 | 1.2 | -- | 3.9 |
| | Mean: | | 0 | 0.24 | 0.97 | 1.75 | 2.80 | 3.08 |
| | s: | | | ±0.05 | ±0.07 | ±0.45 | ±0.17 | ±0.91 |
| | RSD, %: | | | 20.8 | 7.1 | 25.6 | 6.1 | 29.5 |
| | WSF, %: | | | 8 | 32 | 57 | 91 | |
| B | 0 | 20 | 0 | 0.75 | 1.0 | 2.0 | 2.7 | 2.6 |
| | 168 | 168 | 0 | 0.84 | 1.0 | 1.8 | 2.0 | 2.4 |
| | 192 | 20 | 0 | 0.40 | 1.2 | 2.0 | -- | 3.2 |
| | 336 | 168 | 0 | 0.39 | 0.77 | 1.6 | -- | 2.5 |
| | Mean: | | 0 | 0.60 | 0.99 | 1.85 | 2.35 | 2.68 |
| | s: | | | ±0.23 | ±0.16 | ±0.18 | ±0.51 | ±0.37 |
| | RSD, %: | | | 38.3 | 16.2 | 9.8 | 22.0 | 13.8 |
| | WSF, %: | | | 22 | 36 | 68 | 86 | |

The mortality results, compiled in Table 32, correlate well with WSF of JP-8 concentrations. There were no deaths in the controls, 5% mortality at ~ 0.4 mg WSF of shale JP-8/ℓ. At 1.8 mg WSF of shale JP-8/ℓ there was ~ 75% mortality and there was 100% mortality at a mean concentration of 2.55 mg WSF of shale JP-8/ℓ. The 336-hr LC 50 was calculated to be 1.45 mg WSF of shale JP-8/ℓ with 95% confidence limits of 1.24 and 1.70 mg WSF of shale JP-8/ℓ.

### Discussion

Fish exposed to WSF of shale JP-8 exhibited a somewhat different stress response than fish exposed to WSF of JP-4. In WSF of shale JP-8 a much slower death was preceded by pronounced symptoms of discoloration, swimming disorientation, collapsed posterior body, and in some instances, "popeye." Popeye, or exophthalmos, is a condition of lesion and gross enlargement of the eye with symptoms including gas bubble formation and/or hemorrhaging. Fish in WSF of JP-4 died rapidly after the appearance of discoloration and disorientation symptoms. The WSF of shale JP-8 appeared to be far more toxic than the WSF of JP-4 based on the 168-hr LC 50 of WSF of JP-4 to stickleback of 6.8 mg/ℓ compared with the 168-hr 100% mortality of WSF of shale JP-8 at a mean concentration of 2.55 mg/ℓ.

## CONTINUOUS-FLOW BIOASSAY – AUFWUCHS EXPOSED TO WSF OF SHALE JP-8

### Introduction

A 504-hr (21-day) continuous-flow bioassay was conducted to determine the effect of WSF of shale JP-8 on aufwuchs development and to determine the no-effect concentration.

### Procedure

An aufwuchs rack containing 7 substrates was suspended 18 cm below the water surface of each of the 10 test tanks. After 504 hours, 6 substrates were analyzed for metabolic activity, biomass, and chlorophyll. The aufwuchs from the seventh substrate was preserved for future identification of individual species.

The following duplicate volumetric dilutions of solubilizer product were selected for this experiment: 0% (control), 24%, 56%, 75%, and 100%.

The two solubilizers were operated for 20 hr prior to the commencement of the experiment and were thoroughly cleaned and renewed with fresh fuel at 168-hr intervals. Daily maintenance included flow rate calibration and the removal of debris and extraneous algal growth.

WSF of shale JP-8 concentrations were measured 6 times by gas chromatography. Samples were taken at fuel ages of 20 and 168 hr to establish average concentrations for each of the three 168-hr fuel renewal periods. Salinity, temperature, pH, and DO were measured daily while alkalinity, chloride, and TDS were analyzed weekly.

## TABLE 32

### TOXICITY OF SHALE WSF OF JP-8 TO STICKLEBACK

| Dilution of Solubilizer Product, % | Tank Bank | WSF of Shale JP-8 Concn., mg/ℓ | Number of Test Organisms | Cumulative Number of Test Organisms Dead After Hour: | | | | | | | | | | | | | | Mortality % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 24 | 48 | 72 | 96 | 120 | 144 | 168 | 192 | 216 | 240 | 264 | 288 | 312 | 336 | |
| 0 | A | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | B | 0 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | A | 0.24 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 5 |
| 18 | B | 0.60 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 5 |
| 32 | A | 0.99 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 10 |
| 32 | B | 0.99 | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 2 | 2 | 3 | 4 | 20 |
| 65 | A | 1.8 | 20 | 0 | 0 | 0 | 0 | 7 | 7 | 8 | 8 | 8 | 9 | 9 | 13 | 15 | 15 | 75 |
| 65 | B | 1.8 | 20 | 0 | 0 | 0 | 1 | 10 | 11 | 12 | 12 | 12 | 12 | 13 | 13 | 14 | 14 | 70 |
| 100 | A | 2.8 | 20 | 0 | 1 | 4 | 19 | 19 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 100 |
| 100 | B | 2.3 | 20 | 0 | 1 | 5 | 18 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 100 |

## Results

All the Bay water characteristics (Table 33 ) were lower than normal with the sharpest decreases observed in salinity, chloride, and TDS due to high fresh water inflow from two major storms that occurred just before the study commenced. During the experimental period, there was clear, sunny weather favorable for aufwuchs growth; however, the temperature was lower than optimum.

TABLE 33

ANALYSIS OF SAN FRANCISCO BAY WATER USED IN CONTINUOUS-FLOW BIOASSAY OF AUFWUCHS EXPOSED TO WSF OF SHALE JP-8

| Parameter | Value |
|---|---|
| Salinity, °/oo | 11.5 ± 4.1 |
| Temperature, °C | 12.3 ± 3.7 |
| pH | 7.85 ± 0.26 |
| DO, mg/ℓ | 8.3 ± 1.4 |
| Alkalinity, mg as $CaCO_3$/ℓ | 107 ± 22.0 |
| Chloride, g/ℓ | 6.2 ± 3.9 |
| TDS, g/ℓ | 8.8 ± 4.5 |

GC analysis of the WSF of shale JP-8 concentrations (Table 34 ) showed a regular concentration gradient with fuel dilution; duplication between the two banks of tanks was good. The mean of measured WSF of shale JP-8 concentration in Banks A and B, expressed as a percentage of the 100% manifold concentration, were respectively 12 and 15% higher than the selected percentage concentrations. There was no discernible effect of fuel age on fuel concentration.

Results of the biomass, chlorophyll, and productivity measurements, presented in Table 35 , indicate that aufwuchs development was affected at increased WSF of shale JP-8 concentrations. Compared with control aufwuchs, there was a 59% reduction in dry weight in the aufwuchs exposed to the lowest fuel level (0.8 mg/ℓ) and an approximately 90% reduction in dry weight in communities exposed to the three higher concentrations (1.5, 2.1, and 2.3 mg/ℓ). The biomass of the control was slightly higher in organic content ($\simeq$ 5%) than the aufwuchs from the lowest WSF of JP-8 concentration and $\simeq$ 30% higher than the biomass from three higher WSF of JP-8 concentrations.

TABLE 34

WSF OF SHALE JP-8 CONCENTRATIONS FOR AUFWUCHS BIOASSAY
(mg/ℓ)

| Tank Bank | Experimental Time hr | Fuel Age hr | WSF of Shale JP-8 Concentrations at Indicated % Dilution of Solubilizer Product | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 0% | 24% | 56% | 75% | 100% | 100% Manifold |
| A | 0 | 20 | 0 | 0.94 | 1.4 | 2.0 | 2.0 | 2.1 |
| | 168 | 168 | 0 | 1.1 | 1.5 | 2.6 | 2.2 | 2.7 |
| | 192 | 20 | 0 | 0.57 | 1.8 | 2.2 | 2.7 | 2.5 |
| | 336 | 168 | 0 | 0.92 | 1.8 | 2.1 | 2.4 | 2.5 |
| | 360 | 20 | 0 | 0.44 | 1.3 | 1.7 | 2.4 | 2.4 |
| | 504 | 168 | 0 | 0.85 | 1.4 | 2.0 | 2.0 | 2.2 |
| | Mean: | | 0 | 0.80 | 1.5 | 2.1 | 2.2 | 2.4 |
| | s: | | | ±0.24 | ±0.24 | ±0.28 | ±0.17 | ±0.22 |
| | RSD, %: | | | 30.0 | 16.0 | 13.3 | 7.7 | 9.2 |
| | WSF, %: | | | 33.3 | 63.8 | 87.1 | 93.3 | 100 |
| B | 0 | 20 | 0 | 0.59 | 1.6 | 2.0 | 2.0 | - |
| | 168 | 168 | 0 | 0.92 | 2.0 | 2.6 | 2.7 | 2.5 |
| | 192 | 20 | 0 | 0.73 | 2.0 | 2.5 | 2.7 | 2.7 |
| | 336 | 168 | 0 | 0.94 | 1.9 | 1.8 | 2.4 | 2.2 |
| | 360 | 20 | 0 | 0.93 | 1.5 | 1.8 | 2.4 | 2.5 |
| | 504 | 168 | 0 | 0.61 | 1.9 | 2.3 | 2.1 | - |
| | Mean: | | 0 | 0.79 | 1.8 | 2.2 | 2.4 | 2.5 |
| | s: | | | ±0.16 | ±0.20 | ±0.32 | ±0.27 | ±0.18 |
| | RSD, %: | | | 20.3 | 11.1 | 14.5 | 11.3 | 7.2 |
| | WSF, %: | | | 31.9 | 73.0 | 86.7 | 95.6 | 100 |

TABLE 35

STANDING CROP AND PRODUCTIVITY OF AUFWUCHS COMMUNITIES EXPOSED TO FIVE CONCENTRATIONS OF WSF OF SHALE JP-8 FOR 504 HOURS

| WSF of Shale JP-8 Concn., mg/ℓ | Standing Crop: Biomass | | Standing Crop: Chlorophyll | | | | Gross Photosynthesis mg $O_2$/Auf-hr | $PI_{(wt)}$ mg $O_2$/g Dry Wt-hr | $PI_{(Chl\ a)}$ mg $O_2$/mg Chl a-hr |
|---|---|---|---|---|---|---|---|---|---|
| | Dry Wt. mg/Auf. | Dry Wt. % Organic | Chlorophyll a mg/g Dry Wt. | Chlorophyll b mg/g Dry Wt. | Chlorophyll c mg/g Dry Wt. | Pheophytin a mg/g Dry Wt. | | | |
| 0.0 | 27 | 39 | 21 | 2.6 | 14 | 3.5 | 3.41 | 128 | 6 |
| 0.0 | 24 | 40 | 18 | 1.5 | 12 | 1.1 | 2.49 | 102 | 6 |
| 0.80 | 16 | 38 | 10 | 0.8 | 5.0 | 0 | 1.71 | 108 | 10 |
| 0.79 | 14 | 38 | 11 | 0.7 | 5.1 | 0 | 2.01 | 139 | 13 |
| 1.5 | 2.5 | 30 | 2.9 | 0.9 | 0.2 | 0.7 | 0.09 | 36 | 13 |
| 1.8 | 2.7 | 28 | 3.1 | 1.1 | 0.3 | 0.4 | 0.07 | 26 | 9 |
| 2.1 | 2.4 | 28 | 2.6 | 1.2 | 0.8 | 1.2 | 0.04 | 17 | 7 |
| 2.2 | 2.3 | 26 | 1.0 | 0.7 | 0.4 | 0.2 | 0.18 | 79 | 90 |
| 2.2 | 2.5 | 27 | 0.4 | 0.3 | 0.1 | 0.3 | -0.07 | -28 | -70 |
| 2.4 | 2.1 | 32 | 1.5 | 0.4 | 0.6 | 0.2 | 0.02 | 9 | 7 |

There was a fuel concentration-related decrease in chlorophyll a that was very similar to the dry weight results (45% reduction at 0.8 mg WSF of shale JP-8/ℓ and 85–95% reduction at the higher fuel concentrations). The effect of fuel on chlorophyll c was even more marked with 60% and > 95% reduction in the lowest and three highest concentrations, respectively. The chlorophyll b content of aufwuchs exposed to the 4 levels of fuel ranged from 63–83% less than that of the controls. The highest pheophytin a content was observed in control aufwuchs.

The toxic effect of WSF of shale JP-8 on aufwuchs productivity was evident from the gross photosynthesis measurements. Photosynthesis was severely reduced at the three highest WSF of shale JP-8 concentrations. The photosynthetic index with respect to weight $PI_{(wt)}$ was much higher in the aufwuchs from the control and lowest concentrations than in the other three WSF of shale JP-8 concentrations. On the other hand, $PI_{(chl\ a)}$ generally was lower in the control and in the lowest concentration than in the higher concentrations of WSF of shale JP-8, except the highest exposure.

## Discussion

This experiment demonstrated that aufwuchs were surprisingly sensitive to WSF of shale JP-8. Concentrations greater than 1.5 mg WSF of shale JP-8/ℓ were extremely detrimental to aufwuchs, with most indices of toxicity indicating a reduction of approximately 90% in growth and viability. At the lowest WSF of shale JP-8 concentration (0.80 mg/ℓ) there was a 40% reduction in growth and 40–60% reduction in chlorophyll concentrations; however, gross photosynthesis was only slightly affected and on a per gram basis (photosynthetic indices) the growth was judged to be somewhat more productive than the controls. Thus, the no-effect level appears to be between 0 and 0.8 mg/ℓ.

The toxicity of WSF of shale JP-8 to aufwuchs appears to be greater than the toxicity of WSF of JP-4.

At 3 mg WSF of JP-4/ℓ toxic effects were not nearly as severe as those produced by 1.5 mg WSF of shale JP-8/ℓ.

# STATIC BIOASSAY – STICKLEBACK EXPOSED TO WSF OF JP-8

## Introduction

A 96-hr static acute bioassay was performed to determine the LC 50 of the WSF of JP-8 to stickleback and to estimate the WSF of JP-8 concentrations required for a continuous-flow bioassay.

## Procedure

The fuel concentrations selected were the following % dilutions of fuel solubilizer product: 0% (control), 18%, 32%, 65%, 87%, and 100%. Each concentration was prepared in duplicate and renewed daily.

WSF of shale JP-8 concentrations were measured by GC analysis at the beginning and end of one 24-hr experimental period to establish initial WSF of JP-8 concentration and to determine the fuel concentration depletion occurring before fuel renewal.

Stickleback (mean length, 38.5 ± 1.3 mm; mean wet weight, 0.44 ± 0.06 g) were acclimated to Bay water 12 days prior to the start of the experiment. Salinity and temperature were 16.5 ± 5.1 °/oo and 16.4 ± 0.8 °C, respectively. Ten fish each were exposed to 10 ℓ of toxicant contained in 5-gal (19.5-ℓ) glass jars. The solutions were gently aerated and protected by an aluminum foil cover over the glass jars.

Salinity, temperature, DO, and pH were measured daily during the experimental period. Alkalinity, chloride, and TDS were measured at 0 and 96 hr. Fish mortality was recorded at 24-hr intervals.

## Results

Bay water measurements are compiled in Table 36. Salinity was far lower than the seasonal norm (12.1 °/oo compared with 25.0 °/oo in the previous spring).

Because of hot weather, unusually high water temperatures (25.1 °C) were experienced. The large standard deviations in the alkalinity, chloride, and TDS measurements reflected the fluctuating Bay water conditions encountered during the experiment.

TABLE 36

BAY WATER QUALITY DURING STICKLEBACK STATIC BIOASSAY ON WSF OF JP-8

| Parameter | Value |
|---|---|
| Salinity, °/oo | 12.1 ± 6.8 |
| Temperature, °C | 25.1 ± 3.6 |
| pH | 8.1 ± 0.1 |
| DO, mg/ℓ | 9.3 ± 1.9 |
| Alkalinity, mg $CaCO_3$/ℓ | 78.0 ± 76.4 |
| Chloride, g/ℓ | 4.6 ± 5.7 |
| TDS, g/ℓ | 9.9 ± 12.6 |

GC analytical results (Table 37) indicate the initial (Δ0) and the final (Δ24) concentrations of the WSF of JP-8. The 100% WSF concentration was approximately 5.7 mg/ℓ. The measured concentrations were in close agreement with the selected (nominal) volumetric dilutions of WSF of JP-8.

TABLE 37

MEASURED WSF OF JP-8 CONCENTRATIONS DURING STATIC STICKLEBACK BIOASSAY

| Nominal WSF of JP-8 Concn., % | Measured Concentration of WSF of JP-8, mg/ℓ | | | | Bank A and B Mean Measured % WSF of Fuel | | Bank A and B Measured Loss of WSF of JP-8 Δ0 - Δ24 % |
|---|---|---|---|---|---|---|---|
| | Bank A | | Bank B | | | | |
| | Δ0 hr | Δ24 hr | Δ0 hr | Δ24 hr | Δ0 hr | Δ24 hr | |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 | 1.3 | 0.93 | 1.5 | 0.18 | 25 | 10 | 60 |
| 32 | 2.0 | 1.4 | 1.8 | 1.2 | 34 | 24 | 32 |
| 65 | 3.4 | 2.5 | 3.1 | 2.5 | 57 | 44 | 23 |
| 87 | 5.0 | 3.1 | 5.8 | 3.6 | 95 | 59 | 38 |
| 100 | 5.6 | 4.0 | 5.7 | 3.4 | 100 | 65 | 35 |

The mean measured loss of WSF of JP-8 of tank banks A and B during the 24-hr period between fuel renewal ranged from 23 to 60%.

Fish mortality results are presented in Table 38. No LC 50 could be calculated since highest concentration did not kill 50%.

The surviving fish in the two highest concentrations showed stress symptoms, such as discoloration, sluggish movement, and swimming at a 45 deg. angle to the surface. The fish in the lower concentration ranges appeared to be normal. The two deaths in this range were attributed to temperature stress.

Based on these results the same fuel concentrations appear appropriate for the continuous-flow bioassay.

## CONTINUOUS-FLOW BIOASSAY – STICKLEBACK EXPOSED TO WSF OF JP-8

### Introduction

A 14-day study of stickleback exposed to WSF of JP-8 was conducted to determine the LC 50 and the no-effect level.

TABLE 38

ACUTE TOXICITY OF WSF OF JP-8 TO STICKLEBACK (STATIC BIOASSAY)

| WSF of JP-8 | | Cumulative Mortality After Hour: | | | | % |
|---|---|---|---|---|---|---|
| Nominal % | mg/ℓ | 24 | 48 | 72 | 96 | Mortality |
| 0 A | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 B | 0 | 0 | 0 | 0 | 0 | 0 |
| 18 A | 1.28 | 0 | 0 | 0 | 0 | 0 |
| 18 B | 1.50 | 0 | 0 | 0 | 1 | 10 |
| 32 A | 1.98 | 0 | 0 | 0 | 1 | 10 |
| 32 B | 1.85 | 0 | 0 | 0 | 0 | 0 |
| 65 A | 3.37 | 0 | 0 | 0 | 0 | 0 |
| 65 B | 3.08 | 0 | 0 | 0 | 0 | 0 |
| 87 A | 4.99 | 0 | 0 | 0 | 2 | 20 |
| 87 B | 5.83 | 0 | 0 | 0 | 0 | 0 |
| 100 A | 5.61 | 0 | 0 | 0 | 4 | 40 |
| 100 B | 5.72 | 1 | 1 | 1 | 2 | 20 |

## Procedure

Stickleback (mean length 36.7 ± 2.5 mm and mean wet weight 0.38 ± 0.09 g) were acclimated for 7 days to San Francisco Bay water. During the acclimation period the salinity was 22.0 ± 3.7 °/oo and the temperature was 18.7 ± 3.3°C.

Thirteen fish each were exposed to the following duplicate volumetric concentration of WSF of JP-8: 0% (control), 18%, 42%, 75%, and 100%. The fuel concentrations in the exposure tanks were analyzed by GC at fuel ages of 18, 90, 114, 162, and 186 hr. Daily measurements were taken of salinity, temperature, pH, and DO. Alkalinity, chloride, and TDS were analyzed at 0, 168, and 336 hr.

## Results

The water quality analyses of the Bay water (Table 39) indicate the salinity, chloride, and temperature were at levels typical of a late Spring season. There was less fresh water inflow to the Bay than earlier in the Spring.

TABLE 39

BAY WATER PHYSICAL AND CHEMICAL CHARACTERISTICS

| Parameter | Value |
|---|---|
| Salinity, °/oo | 23.2 ± 2.6 |
| Temperature, °C | 15.5 ± 4.5 |
| pH | 8.0 ± 0.2 |
| DO, mg/ℓ | 9.8 ± 2.3 |
| Alkalinity, mg as $CaCO_3$ | 108.7 ± 9.2 |
| Chloride, g/ℓ | 13.4 ± 1.1 |
| TDS, g/ℓ | 39.9 ± 9.6 |

The results of GC analyses (Table 40) indicate a regular concentration gradient in both banks of exposure tanks. The goodness of duplication may be discerned by the 4–9% difference between the means of replicate WSF of JP-8 dilutions in the two banks of tanks. The 100% fuel solubilizer product concentration averaged 4.8 mg WSF of JP-8/ℓ. The concentration was affected by fuel age – at ages of ≤ 114 hr the concentration was > 5 mg WSF of JP-8/ℓ, but at ages of ≥ 162 hr the concentration decreased to approximately 4 mg WSF of JP-8/ℓ.

The greatest difference between measured and selected fuel concentration in the exposure tanks (approximately 15–20%) occurred at the two higher exposure levels. There was little difference (approximately 2–6%) at the lower exposure levels.

The replication of fuel concentration between the two banks of tanks was satisfactory (% RSD ~15–30%) at exposure levels of 42%, 75%, 100%, and 100% fuel solubilizer product. Fluctuations in concentration can result from volatility and biodegradation which in turn are affected by fish activity and metabolic products. The lowest fuel level (18% of solubilizer product) was not well replicated between the two banks of tanks (% RSD of 75% and 126%). This poor replication was most likely due to a variety of factors including volatility, biodegradation, difficulty in maintaining the selected dilution, and analytical error at the low WSF of JP-8 concentrations near the limit of detection. With a relatively non-volatile fuel (such as shale JP-8) the limit of analytical detection was improved by concentrating pentane extracts to very small volumes (0.1–0.5 mℓ). With the more volatile WSF of JP-8 this approach was unworkable; experience had shown that pentane extracts of WSF of JP-8 that were reduced to less than 3 mℓ rapidly lost volatile components and the detectability became poorer.

The mortality results, compiled in Table 41, correlate well with WSF of JP-8 concentrations. The 336-hr LC 50 was 3.22 mg WSF of JP-8/ℓ with 95% confidence limits of 2.75 and 3.76 mg WSF of JP-8/ℓ. The no-effect

TABLE 40

MEASURED WSF OF JP-8 CONCENTRATIONS FOR STICKLEBACK BIOASSAY (CONTINUOUS FLOW)

| Tank Bank | Experimental Time hr | Fuel Age hr | WSF of JP-8 Concentrations (mg/ℓ) at Indicated % Dilutions of Solubilizer Product | | | | | 100% (Fuel Solubilizer Product) |
|---|---|---|---|---|---|---|---|---|
| | | | 0% | 18% | 42% | 75% | 100% | |
| A | 0 | 18 | 0 | 0.13 | 2.6 | 2.9 | 3.8 | - |
| | 96 | 114 | 0 | 1.2 | 2.1 | 3.1 | 4.0 | 5.4 |
| | 168 | 186 | 0 | 0.12 | 0.94 | 2.9 | 3.0 | 4.3 |
| | 192 | 18 | 0 | 1.5 | 2.7 | 3.9 | 5.0 | 5.3 |
| | 264 | 90 | 0 | 1.2 | 2.0 | 3.3 | 4.6 | 5.0 |
| | 336 | 162 | 0 | 0.59 | 1.7 | 2.4 | 3.0 | 3.9 |
| | Mean: | | 0 | 0.79 | 2.0 | 3.1 | 3.9 | 4.8 |
| | s: | | | ±0.59 | ±0.64 | ±0.50 | ±0.82 | ±0.65 |
| | RSD, %: | | | 75 | 32 | 16 | 21 | 14 |
| | WSF, %: | | 0 | 17 | 42 | 65 | 82 | 100 |
| B | 0 | 18 | 0 | 0.07 | 1.7 | 2.9 | 3.8 | 5.0 |
| | 96 | 114 | 0 | 0.02 | 1.2 | 2.8 | 3.4 | 5.5 |
| | 168 | 186 | 0 | 0.09 | - | 2.1 | 3.3 | 3.8 |
| | 192 | 18 | 0 | 1.1 | 2.6 | 3.5 | 4.7 | 5.4 |
| | 264 | 90 | 0 | 0.89 | 2.2 | 2.9 | 4.1 | 5.7 |
| | 336 | 162 | 0 | 0.09 | 1.5 | 2.7 | 3.2 | 4.0 |
| | Mean: | | 0 | 0.38 | 1.8 | 2.8 | 3.8 | 4.9 |
| | s: | | | ±0.48 | ±0.56 | ±0.45 | ±0.58 | ±0.81 |
| | RSD, %: | | | 126 | 31 | 16 | 15 | 17 |
| | WSF, %: | | 0 | 8 | 38 | 58 | 77 | 100 |

TABLE 41

TOXICITY OF WSF OF JP-8 TO STICKLEBACK
(CONTINUOUS FLOW)

| WSF of JP-8 Concn. mg/ℓ | No. of Test Organisms | Cumulative Number of Test Organisms Dead After Hour: | | | | | | | | | | | | | | % Mortality |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 24 | 48 | 72 | 96 | 120 | 144 | 168 | 192 | 216 | 240 | 264 | 288 | 312 | 336 | |
| 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 7.7 |
| 0.79 | 13 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 3 | 23.1 |
| 0.38 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2.01 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 7.7 |
| 1.86 | 13 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 15.4 |
| 3.08 | 13 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 3 | 4 | 4 | 5 | 5 | 5 | 5 | 38.5 |
| 2.82 | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 46.2 |
| 3.90 | 13 | 0 | 4 | 4 | 4 | 6 | 7 | 8 | 8 | 8 | 9 | 10 | 10 | 10 | 10 | 76.9 |
| 3.76 | 13 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 3 | 3 | 4 | 7 | 7 | 8 | 8 | 61.5 |

level appeared to be between 0 and 0.8 mg WSF of JP-8/ℓ. A 168-hr LC 50 could not be calculated because 50% mortality was not reached at that time.

### Discussion

Fish responded slowly to the effects of WSF of JP-8 as evidenced by the fact that there were few deaths during the first week and then the mortality gradually increased during the second week of the experiment. Fish behavior was abnormal (sluggishness) at the two highest exposure levels (3.0 and 3.8 mg WSF of JP-8/ℓ). Discoloration, a stress symptom, was also observed at the highest level.

## CONTINUOUS FLOW BIOASSAY – AUFWUCHS EXPOSED TO WSF OF JP-8

### Introduction

A 336-hr continuous-flow bioassay was conducted to determine the effect of WSF of JP-8 on aufwuchs development and to determine the no-effect concentration.

### Procedure

Aufwuchs were exposed to the following duplicate volumetric dilutions of solubilizer product: 0% (control), 18%, 32%, 65%, and 87%. The two solubilizers were operated for 20 hr prior to the commencement of the experiment and thoroughly cleaned and renewed with fresh fuel at 168 hr.

An aufwuchs rack containing 7 growth units was suspended 18 cm below the water surface of each of the test tanks. After 336 hr, 6 growth units were analyzed for biomass, chlorophyll, and productivity. The seventh substrate was preserved for future identification of individual species.

WSF of JP-8 concentrations were measured by GC 6 times during the experiment. Samples were taken at the following fuel ages: 20 hr (twice), 92 hr, 116 hr, 164 hr, and 188 hr. Salinity, temperature, pH, and DO were measured daily while alkalinity, chloride, and TDS were analyzed at 0, 168, and 336 hr.

### Results

Table 42 indicates that the salt content of the Bay water was higher than in the preceding aufwuchs study. The Bay water composition was typical of early summer values.

GC analysis of the WSF of JP-8 concentrations (Table 43) indicated a regular concentration gradient with fuel dilution from a mean solubilizer product concentration of 4.7 mg/ℓ. Greater differences between nominal and measured fuel concentrations occurred at the higher exposure levels. The mean difference was 17% lower at the 87% dilution and 8% lower at the 65% dilution, but there was only a 5% decrease at the two lower dilutions (18% and 32%). As in preceding studies with WSF of JP-8, the differences in WSF of

TABLE 42

ANALYSIS OF SAN FRANCISCO BAY WATER USED IN CONTINUOUS-FLOW BIOASSAY OF AUFWUCHS IN WSF OF JP-8

| Parameter | Value |
|---|---|
| Salinity, °/oo | 24.6 ± 1.9 |
| Temperature, °C | 15.4 ± 3.9 |
| pH | 8.2 ± 0.4 |
| DO, mg/ℓ | 9.9 ± 2.4 |
| Alkalinity, mg $CaCO_3$/ℓ | 119.3 ± 18.8 |
| Chloride, g/ℓ | 12.7 ± 0.9 |
| TDS, g/ℓ | 26.5 ± 11.8 |

JP-8 in replicate banks of tanks was highest (% RSF > 100%) at the lowest (18%) dilution for the reasons set forth previously.

Results of biomass, chlorophyll and productivity measurements (Table 44) indicate that aufwuchs development was adversely affected by increasing concentrations of WSF of JP-8. The most pronounced effect was on biomass, which was lower than the control by 23% at the two lowest concentrations (0.7 and 1.4 mg WSF of JP-8/ℓ) and was decreased by 64% with respect to the control at the two highest concentrations (2.7 and 3.3 mg WSF of JP-8/ℓ). Organic content was affected only at 3.3 mg WSF of JP-8/ℓ as indicated by a 17% decrease compared with control aufwuchs. No dose-response trends were observed with respect to the three chlorophyll pigments. Pheophytin *a* was not present in any aufwuchs except for a trace in one of the controls.

The toxicity of WSF of JP-8 to aufwuchs productivity was evident in the gross photosynthesis measurements. There were decreases of approximately 50% at the three highest WSF of JP-8 concentrations (1.4, 2.7, and 3.3 mg/ℓ), but the lowest WSF of JP-8 concentration of 0.7 mg/ℓ indicated approximately the same productivity as the control. Aufwuchs viability expressed by $PI_{(wt)}$ and $PI_{(chl\ a)}$ decreased with increasing fuel exposures.

The no-effect level of WSF of JP-8 on aufwuchs is in the range 0.7 to 1.4 mg/ℓ when both biomass and productivity are considered. For biomass alone, the no-effect level is higher (in the range of 2.7 to 3.3 mg WSF of JP-8/ℓ).

## Discussion

The toxic effect of WSF of JP-8 on aufwuchs is a retardation of total growth (biomass) rather than the inhibition of pigment formation or suppression of respiration rate. On a weight basis, the fuel had no effect on chlorophyll

TABLE 43

MEASURED WSF OF JP-8 CONCENTRATIONS FOR AUFWUCHS BIOASSAY (CONTINUOUS FLOW)

| Tank Bank | Experimental Time hr | Fuel Age hr | WSF of JP-8 Concentrations (mg/ℓ) at Indicated % Dilutions of Solubilizer Product | | | | | 100% (Fuel Solubilizer Product) |
|---|---|---|---|---|---|---|---|---|
| | | | 0% | 18% | 32% | 65% | 87% | |
| A | 0 | 20 | 0 | 1.0 | 1.9 | 3.0 | 3.9 | 4.8 |
| | 96 | 116 | 0 | 0.04 | 0.15 | 1.5 | 3.3 | - |
| | 168 | 188 | 0 | 0.08 | 0.17 | 1.8 | 2.4 | 4.3 |
| | 192 | 20 | 0 | 1.1 | 1.6 | 2.3 | 3.2 | 4.4 |
| | 264 | 92 | 0 | 1.2 | 1.8 | 2.7 | 2.8 | 5.5 |
| | 336 | 164 | 0 | 1.1 | 2.4 | 3.1 | 3.6 | 5.1 |
| | Mean: | | 0 | 0.75 | 1.3 | 2.4 | 3.2 | 4.8 |
| | s: | | | ±0.54 | ±0.95 | ±0.65 | ±0.54 | ±0.50 |
| | RSD, %: | | | 72 | 73 | 27 | 17 | 10 |
| | WSF, %: | | 0 | 16 | 28 | 50 | 66 | 100 |
| B | 0 | 20 | 0 | 2.1 | 1.8 | 2.8 | 4.2 | 4.8 |
| | 96 | 116 | 0 | 0.07 | 1.6 | 3.5 | 3.8 | 3.9 |
| | 168 | 188 | 0 | 0.09 | 0.2 | - | 2.5 | 3.7 |
| | 192 | 20 | 0 | 1.0 | 2.0 | 2.7 | 3.3 | 4.9 |
| | 264 | 92 | 0 | 0.13 | 1.6 | 2.1 | 3.0 | 6.3 |
| | 336 | 164 | 0 | 0.17 | 1.7 | 3.6 | 3.4 | 3.9 |
| | Mean: | | 0 | 0.59 | 1.5 | 2.9 | 3.4 | 4.6 |
| | s: | | | ±0.82 | ±0.65 | ±0.62 | ±0.60 | ±0.98 |
| | RSD, %: | | | 139 | 43 | 21 | 18 | 21 |
| | WSF, %: | | 0 | 13 | 33 | 64 | 74 | 100 |

TABLE 44

STANDING CROP AND PRODUCTIVITY OF AUFWUCHS COMMUNITIES EXPOSED TO FIVE CONCENTRATIONS OF WSF OF JP-8 FOR 336 HOURS

| WSF of JP-8 Concn. mg/ℓ | Standing Crop | | | | | | Gross Photosynthesis mg $O_2$/Auf-hr | $PI_{(wt)}$ mg $O_2$/g Dry wt-hr | $PI_{(Chl\ a)}$ mg $O_2$/mg Chl a-hr |
|---|---|---|---|---|---|---|---|---|---|
| | Biomass | | Chlorophyll | | | | | | |
| | Dry Wt. mg/Auf. | % Organic | Chlorophyll a mg/g Dry Wt. | Chlorophyll b mg/g Dry Wt. | Chlorophyll c mg/g Dry Wt. | Pheophytin a mg/g Dry Wt. | | | |
| 0.0 | 38 | 46 | 3.5 | 0.21 | 1.7 | 0 | 1.46 | 38 | 11 |
| 0.0 | 54 | 45 | 4.5 | 0.29 | 2.2 | 0.03 | 1.63 | 30 | 7 |
| 0.77 | 32 | 43 | 5.3 | 0.31 | 2.6 | 0 | 1.63 | 51 | 10 |
| 0.59 | 35 | 45 | 4.2 | 0.25 | 2.0 | 0 | 1.13 | 32 | 8 |
| 1.33 | 38 | 46 | 3.6 | 0.20 | 1.7 | 0 | 1.38 | 36 | 10 |
| 1.50 | 35 | 46 | 3.9 | 0.22 | 1.8 | 0 | 0.36 | 10 | 3 |
| 2.40 | 17 | 43 | 4.2 | 0.22 | 1.5 | 0 | 0.46 | 27 | 7 |
| 2.95 | 23 | 46 | 3.8 | 0.18 | 1.6 | 0 | 0.36 | 16 | 4 |
| 3.20 | 14 | 38 | 5.8 | 0.33 | 2.4 | 0 | 0.49 | 34 | 6 |
| 3.37 | 11 | 36 | 5.0 | 0.33 | 1.5 | 0 | 0.25 | 22 | 4 |

pigments content; in fact, in some instances there were higher concentrations of the chlorophylls at the higher fuel exposure levels. The same pattern of response was observed for productivity as demonstrated by the $PI_{(wt)}$. Several explanations can be put forth to account for this phenomenon. The control aufwuchs may have reached maximum growth and been in a declining phase of chlorophyll viability. More likely the controls were more advanced in terms of species diversity with inclusion of non-chlorophyll bearing species in the community. A third possibility is that aufwuchs exposed to fuel were initially inhibited, but became acclimated and more viable in the period when sampling occurred.

## DISCUSSION

### INTRODUCTION

From the results of current and previously reported work, the relative toxicity of the jet fuels JP-4, JP-8, shale JP-8, and JP-9 and its components RJ-4, RJ-5, and methylcyclohexane (MCH) to rainbow trout and flagfish can be assessed. Similarly, the relative toxicity of these fuels (except JP-9) to stickleback and aufwuchs can be evaluated from current work.

### RELATIVE TOXICITY OF HYDROCARBON FUELS TO FLAGFISH AND RAINBOW TROUT

#### Sublethal Effects

Sublethal effects of WSF's of JP-4, JP-8, and shale JP-8 were assessed by measuring the growth rate of fry and the egg hatching rate. The WSF of JP-9 and its components were included in comparisons of fuel accumulation and the success of egg hatching.

The no-effect level with respect to growth for WSF of JP-8 on rainbow trout was $< 1.4$ mg/$\ell$ (the lowest level tested); for WSF of JP-4 the no-effect level was 1.1 mg/$\ell$; for WSF of shale JP-8 the no-effect level was $< 0.13$ mg/$\ell$ (the lowest level tested). The conclusion is that shale JP-8 is the most toxic of these three fuels, and JP-4 and JP-8 appeared to be in the same range of toxicity to rainbow trout.

Flagfish growth was unaffected by the presence of 1.7 mg WSF of JP-8/$\ell$; however, $1.5 \pm 0.8$ mg WSF of JP-4/$\ell$ retarded flagfish growth. The predicted no-effect level for WSF of JP-4 on flagfish growth was $0.6 \pm 0.2$ mg/$\ell$. This is in a similar range with WSF of shale JP-8 which had a no-effect level of 0.51 to 1.35 mg/$\ell$. The conclusion is that JP-8 is the least toxic substance and JP-4 and shale JP-8 are approximately equal in toxicity to flagfish.

The effect of fuels on rate of egg hatching is species related; the rate of egg hatching is retarded in flagfish and accelerated in rainbow trout. Retardation effect on flagfish eggs were observed at concentrations $\geq 2.7$ mg WSF of JP-4/$\ell$, $\geq 1.7$ mg WSF of JP-8/$\ell$, and $\geq 0.20$ mg WSF of shale JP-8/$\ell$. Acceleration effects on rainbow trout eggs were observed at concentrations $\geq 2.1$ mg WSF of JP-8/$\ell$, $\geq 1.1$ mg WSF of JP-4/$\ell$, and $\geq 0.60$ mg WSF of

shale JP-8/ℓ. The conclusion is that WSF of shale JP-8 is the most toxic of the three fuels to eggs of both species, and the order of toxicity of WSF's of JP-4 and JP-8 to eggs is species dependent.

The success of rainbow trout egg hatching was unaffected in the range of concentrations examined for WSF of JP-4 (0–6.1 mg/ℓ), WSF of JP-8 (0–8 mg/ℓ), and WSF of shale JP-8 (0–1.35 mg/ℓ).

The success of flagfish egg hatching was unaffected in the range of concentrations examined for WSF of JP-4 (0–6.6 mg/ℓ, November 1978 Third Annual Report AMRL-TR-78-65) and WSF of shale JP-8 (0–1.35 mg/ℓ). For WSF of JP-8 there was no effect in the range 0–3.3 mg/ℓ, but eggs were not hatched to viable fry at a concentration of 6.8 mg/ℓ (November 1977 Second Annual Report AMRL-TR-77-54). The WSF of JP-9 was more toxic than the other fuels to flagfish eggs. A concentration in excess of 0.23 mg/ℓ reduced hatching success. This toxicity was ascribed to the RJ-5 component of JP-9, since it was found that an RJ-5 concentration of greater than 0.05 mg/ℓ reduced the hatching success of flagfish eggs (June 1976 Annual Report AMRL-TR-76-50).

Accumulation of fuels in whole-body rainbow trout fish tissues occurred at all aqueous fuel concentrations measured. The WSF of JP-4 mean accumulation ratio was 170, compared with mean ratios of 88 for WSF of JP-8 (November 1979 Fourth Annual Report AMRL-TR-79-70) and 72 for WSF of shale JP-8. The WSF of MCH accumulated to a similar extent as the WSF of JP-4; the accumulation ratio was 150. However, the WSF's of both RJ-4 and RJ-5 accumulated to much greater extents – rainbow trout concentrated RJ-4 9800 times and RJ-5 3900 times (June 1976 Annual Report AMRL-TR-76-50).

Lethal Effects

Rainbow trout survival was influenced more by WSF of shale JP-8 than by either WSF of JP-4 or WSF of JP-8. There was a significant effect on rainbow trout mortality at WSF of shale JP-8 concentrations between 0.13 and 0.32 mg/ℓ, at WSF of JP-8 concentrations between 1.4 and 1.8 mg/ℓ and at WSF of JP-4 concentrations between 1.7 and 3.5 mg/ℓ. Two of the components of JP-9 were more lethal to rainbow trout survival than shale JP-8. Non-lethal concentrations were below 0.03 mg/ℓ RJ-4, below 0.04 mg/ℓ RJ-5, below 0.80 mg/ℓ MCH, and below 0.38 mg/ℓ WSF of JP-9.

Flagfish survival was influenced most by WSF of shale JP-8, next by WSF of JP-8, and least by WSF of JP-4. Flagfish mortality increased at WSF of shale JP-8 concentrations between 0.51 and 1.35 mg/ℓ, by WSF of JP-8 concentrations between 1.5 and 3.0 mg/ℓ, and by WSF of JP-4 concentrations above 3.0 mg/ℓ.

Based on the preceding comparisons, both sublethal and lethal effects indicate that the WSF of shale JP-8 is far more toxic than either WSF of JP-4 or WSF of JP-8. No definitive statement can be made as to the order of toxicity of WSF of JP-4 and JP-8. The order is mixed for these two fuels suggesting that they are in the same general range of toxicity to fresh water fish.

Even more toxic than the WSF of shale JP-8 are two of the components of JP-9: RJ-4 and RJ-5 (June 1976 Annual Report AMRL-TR-76-50). The MCH component appears to be slightly less toxic than the WSF of shale JP-8.

## RELATIVE TOXICITY OF HYDROCARBON FUELS TO STICKLEBACK AND AUFWUCHS

### Stickleback

The relative toxicity of the three hydrocarbon fuels (JP-4, JP-8, and shale JP-8) to stickleback is summarized in Table 45. Shale JP-8 appears to be the most toxic fuel of the three. However, its toxicity may have been enhanced by the low salinity (10 °/oo) during the shale JP-8 bioassay compared with higher salinities (23 °/oo and 29 °/oo) measured in the JP-8 and JP-4 experiments. Low salinity has been reported to adversely affect biological communities (Pearson et al., 1970).

The WSF of JP-4 appears to be less toxic than the WSF of JP-8, but the evidence is not clearcut. The 168-hr LC 50 tends to support this contention, although there is some doubt because a 168-hr LC 50 value for WSF of JP-8 was not elicited due to less than 50% mortality in the combined duplicate exposure tanks of the highest concentration examined. The highest concentration examined for this fuel was 3.8 mg/ℓ. One of the duplicate exposure tanks did indicate > 50% mortality at this concentration suggesting that it was close to the critical value and therefore well below the 6.8 mg/ℓ 168-hr LC 50 value found for JP-4. Unfortunately, there is no basis for comparison of the three fuels at 336 hr because the JP-4 bioassay had to be terminated after 168 hr.

TABLE 45

RELATIVE TOXICITY OF WSF OF HYDROCARBON FUELS TO STICKLEBACK

(mg/ℓ)

| Parameter | WSF of JP-4 | WSF of Shale JP-8 | WSF of JP-8 |
|---|---|---|---|
| 336-hr LC 50 | - | 1.45 | 3.22 |
| 168-hr LC 50 | 6.8 | 1.95 | >3.8 |
| 336-hr No-Effect | - | 0-0.2 | 0-0.8 |
| 168-hr No-Effect | 1.2-5.1 | 1.0-1.8 | 1.9-2.8 |

The 168-hr no-effect levels are not helpful in assessing the relative toxicity of the fuels because the critical ranges overlap.

Another indication of toxicity is the behavior of surviving fish. At a concentration of 1.8 mg WSF of shale JP-8/ℓ severely stressed behavior was observed, but at 1.9 mg WSF of JP-8/ℓ stress symptoms were barely discernible. Stress symptoms were not observed at 1.4 mg WSF of JP-4/ℓ, but stress was evident at 5.0 mg WSF of JP-4/ℓ (the next highest concentration tested). These observations tend to support the conclusion that shale JP-8 is the most toxic compound.

Aufwuchs

The relative toxicity of the three fuels to aufwuchs is the following: the 504-hr no-effect concentration is 0–3.3 mg WSF of JP-4/ℓ and 0–0.8 mg WSF of shale JP-8/ℓ; the 336-hr no-effect concentration is 0.8–1.4 mg WSF of JP-8/ℓ.

Indications are that WSF of shale JP-8 is the most toxic of the three fuels. The results are not definitive because the lowest fuel concentration of the WSF of JP-4 was 3.3 mg/ℓ, and it is not known at what level between 0 and 3.3 mg/ℓ that an effect occurs. For comparison of the toxicities of WSF of shale JP-8 (504-hr bioassay duration) and WSF of JP-8 (336-hr bioassay duration) the results are not decisive due to the difference in duration. Perhaps if the WSF of JP-8 bioassay had been extended to 504 hr the no-effect level would have decreased to less than the 0.8 mg/ℓ value obtained for shale JP-8. In addition, the salinity was low (12 °/oo during the shale JP-8 bioassay compared with the higher salinities of 25–27 °/oo for the other two fuels.

## RELATIVE TOXICITY OF JET FUELS TO OTHER HYDROCARBON FUELS

### Fresh Water Environment

No work has been reported in the literature on the effects of the WSF of jet fuels or the WSF of refined fuels, but studies have been performed on the WSF of crude oil. This material contains some similar components to those under consideration in this report. Refined oils have been proposed to be more lethal than crude oil, due to a greater proportion of moderately volatile aromatic compounds (Anderson et al. 1974). Erhardt and Blumer (1972) report that aromatic compounds are the most toxic components present in the persistent WSF of oils.

Hedtke and Puglisi (1980) studied the effects of WSF of waste oil on flagfish and reported the following: impaired egg production at 3.4 mg WSF/ℓ; a 30-day egg to adult stage LC 50 of 8.1 mg WSF/ℓ; and a 96-hr continuous-flow LC 50 to adults of 9.5 mg WSF/ℓ. These values are much higher than the jet fuel results. The difference in toxicity may be attributed not only to the difference in components between jet fuels and crude oil but the crude oil work was performed with a modified proportional diluter which cascades the WSF through a series of compartments creating volatility loss. Proportional diluters were used early in the jet fuel studies (June 1976 Annual Report AMRL-TR-76-50) and found unsuitable for volatile materials.

Previous studies with the jet fuels, JP-4 and JP-8 (November 1978 Third Annual Report AMRL-TR-78-65 and November 1977 Second Annual Report AMRL-TR-77-54) have shown a greater accumulation of the WSF occurred in the liver and gastro-intestinal tract than in the muscle tissue of flagfish and golden shiner. The current study did not clearly show a difference between liver and muscle accumulation of WSF of shale JP-8. McKeown and March (1978) reported that WSF of Bunker C oil (refined) was accumulated higher in the liver, kidney, and gill tissues than in the muscle tissue of rainbow trout.

Marine Environment

Most marine studies in the literature deal with crude oil and little information exists on the effects of WSF of refined fuels on estuarine fish. Anderson et al. (1974) found that the 96-hr LC 50's of WSF of No. 2 fuel oil to estuarine fish were 3.9 mg/ℓ to Cyprinodon variegatus and 6.3 mg/ℓ to Menidia beryllina and Fundulus similus. These values were comparable to the estimated 96-hr LC 50's of > 2.6 mg/ℓ and > 5.7 mg/ℓ for the WSF of shale JP-8 and JP-8, respectively, to the stickleback.

The toxicity of WSF of various fuels on microalgae has been investigated by Pulich et al. (1974) and Winters et al. (1976). Concentrations ranging from 0.04 to 0.40 WSF of No. 2 fuel oil were observed to be lethal to diatom Thalassiosira pseudomona (Pulich et al., 1974), and that concentrations > 0.4 mg/ℓ affected photosynthetic activities in green and blue alga species. These values are lower than the no-effect concentrations estimated in the present study since their work involved pure algal cultures. In addition, Pulich et al. (1974) postulated that aromatic compounds are possibly the most toxic of the components of the WSF since these compounds are degraded slowly and with difficulty by fungal and bacterial populations. Winters et al. (1976) also concluded that specific components of the WSF of fuel oils and not necessarily the total amount of WSF were critical to growth of microalgae.

ENVIRONMENTAL IMPACT

The no-effect level of the WSF of the several fuels examined during the past 6 years has been detailed (see relative toxicity – fresh water fish) with respect to survival and growth of the test species, rainbow trout and flagfish. The studies generally indicate that rainbow trout are more sensitive than flagfish. Of the materials studied two of the components of JP-9 (RJ-4 and RJ-5) would appear to have the most deleterious effect on the aquatic environment exhibiting lethal concentrations to rainbow trout in the range of 0.03 to 0.04 mg/ℓ. The RJ-5 component, in particular, appeared to cause the most damage to fish. This component separated out from the fuel mixture when it was subjected to a turbulent water stream. Globules of RJ-5, which is denser than water, can be expected to be deposited on the bottom of streams after a spill and present a more permanent environmental hazard than the other materials studied.

The WSF of shale JP-8 might have serious environmental impact at very low concentrations. Growth effects to rainbow trout were discerned at concentrations of 0–0.13 mg/ℓ and regression analysis gave an estimated no-effect level of 0.06 mg/ℓ.

The no-effect level of the WSF of JP-4 and JP-8 to the test species appeared to be at least an order of magnitude greater than shale JP-8 at levels of 0.6 mg/ℓ and above. Thus, the effects of a fuel spill with these two fuels would be far less likely than shale JP-8 to have widespread environmental consequences.

Another factor to be considered is the relative persistence of these materials. The WSF of both JP-4 and JP-8 are highly volatile compared with the WSF of shale JP-8. All these materials are biodegradable and thus would not persist indefinitely.

To estimate the period of persistence of a fuel in the environment, a series of studies is needed to relate aquatic environmental parameters such as temperature, turbulence, light, water characteristics, etc. to the rate of fuel disappearance due to volatility and/or biodegradation.

Another environmental consequence of fuel spills is the accumulation of fuel components in fish tissue. Accumulation occurred at all aqueous fuel concentrations measured. Thus, the presence of even very low levels of fuel in a body of water would have some impact on fish (e. g. causing off tastes and flavors). The long-term consequences of accumulation are mitigated by depuration. Depuration rates varied for the different fuels, but were similar for WSF of JP-4 and JP-8; a 90% depuration of WSF of JP-8 from flagfish whole-body tissue occurred after 14 days and nearly 100% depuration of WSF of JP-4 from golden shiner innards occurred after 17 days. These data were derived from fish exposed to approximately 1 mg/$\ell$ WSF of fuel. When the fuel was accumulated from a WSF of JP-4 of 2.5 mg/$\ell$, there was an approximately 75% removal from innards after 17 days depuration. MCH was almos' completely voided in 12 hr from rainbow trout tissue, but RJ-4 and RJ-5 wer completely retained in the tissue after 8 days depuration.

APPENDIX 1

TABLE 46

Growth Rate of Rainbow Trout Measured By Length

| | Total days → | 25 | | | 47 | | | 61 | | | 73 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Swim-up day → | 5 | | | 27 | | | 41 | | | 53 | | |
| Tank | WSF of Shale JP-8 mg/ℓ ± s | Length* mm | s** mm | n*** | Length mm | s mm | n | Length mm | s mm | n | Length mm | s mm | n |
| Bank A | | | | | | | | | | | | | |
| 1 | 0 | 23.9 | 0.6 | 22 | 28.3 | 2.8 | 43 | 35.4 | 4.0 | 45 | 39.4 | 4.4 | 44 |
| 2 | 0.13 ± 0.05 | 23.7 | 1.2 | 22 | 27.8 | 2.5 | 41 | 35.0 | 3.9 | 45 | 38.5 | 4.6 | 43 |
| 3 | 0.31 ± 0.10 | 23.0 | 0.9 | 22 | 27.6 | 2.1 | 36 | 33.5 | 3.1 | 40 | 37.3 | 4.5 | 42 |
| 4 | 0.60 ± 0.17 | 23.4 | 1.3 | 22 | 24.5 | 1.6 | 23 | 29.3 | 2.9 | 43 | 35.1 | 3.4 | 40 |
| 5 | 1.25 ± 0.28 | 22.9 | 0.8 | 22 | | | | | | | | | |
| Bank B | | | | | | | | | | | | | |
| 1 | 0 | 22.8 | 1.0 | 29 | 27.0 | 2.3 | 39 | 33.5 | 3.8 | 49 | 39.0 | 3.9 | 39 |
| 2 | 0.12 ± 0.06 | 22.5 | 1.1 | 29 | 25.5 | 2.5 | 37 | 32.6 | 3.2 | 47 | 37.6 | 5.0 | 41 |
| 3 | 0.32 ± 0.13 | 22.4 | 1.0 | 29 | 25.9 | 1.9 | 40 | 31.6 | 3.0 | 47 | 37.4 | 4.0 | 42 |
| 4 | 0.65 ± 0.16 | 22.7 | 0.9 | 29 | 24.9 | 1.9 | 24 | 28.7 | 3.0 | 41 | 34.4 | 4.2 | 43 |
| 5 | 1.22 ± 0.21 | 21.4 | 1.0 | 29 | | | | | | | | | |
| Banks A and B | | | | | | | | | | | | | |
| 1 | 0 | 23.2 | 0.7 | 51 | 27.6 | 2.6 | 82 | 34.4 | 4.0 | 94 | 39.2 | 4.2 | 83 |
| 2 | 0.13 ± 0.06 | 23.0 | 1.3 | 51 | 26.7 | 2.7 | 78 | 33.8 | 3.7 | 92 | 38.1 | 4.8 | 84 |
| 3 | 0.32 ± 0.12 | 22.7 | 1.0 | 51 | 26.7 | 2.1 | 76 | 32.4 | 3.2 | 87 | 37.4 | 4.2 | 84 |
| 4 | 0.63 ± 0.17 | 22.9 | 1.1 | 51 | 24.5 | 1.7 | 47 | 29.0 | 2.9 | 84 | 34.7 | 3.9 | 83 |
| 5 | 1.24 ± 0.25 | 22.0 | 1.1 | 51 | | | | | | | | | |

* The length is the average of the number n measurements.

** s is standard deviation.

*** n is number measured.

APPENDIX 1
TABLE 47

Growth Rate of Rainbow Trout Measured by Length and Weight

| Total Days → | | 94 | | | | | | 119 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Swim-up Day → | | 74 | | | | | | 99 | | | | | |
| WSF of Shale JP-8 | | Length | | | Weight | | | Length | | | Weight | | |
| Nominal % | mg/L ± s | mm | s* | n** | g | s | n | mm | s | n | g | s | n |
| Bank A | | | | | | | | | | | | | |
| 0 | 0 | 52.3 | 4.4 | 47 | 2.140 | 0.573 | 47 | 75.4 | 9.6 | 31 | 5.808 | 2.212 | 31 |
| 6 | 0.13±0.05 | 51.2 | 5.7 | 44 | 2.002 | 0.604 | 44 | 65.7 | 9.6 | 29 | 4.581 | 1.754 | 29 |
| 13 | 0.31±0.10 | 48.3 | 5.7 | 46 | 1.764 | 0.567 | 46 | 65.8 | 7.7 | 25 | 4.498 | 1.516 | 25 |
| 25 | 0.60±0.17 | 41.6 | 4.7 | 44 | 1.175 | 0.406 | 44 | 56.0 | 8.7 | 27 | 2.901 | 1.223 | 27 |
| 50 | 1.25±0.28 | | | | | | | 37.6 | 9.7 | 27 | 1.100 | 0.966 | 27 |
| Bank B | | | | | | | | | | | | | |
| 0 | 0 | 52.4 | 6.4 | 47 | 2.200 | 0.717 | 47 | 71.3 | 9.0 | 28 | 5.281 | 1.710 | 28 |
| 6 | 0.12±0.06 | 50.8 | 6.1 | 48 | 2.035 | 0.660 | 48 | 69.6 | 5 5 | 31 | 5.108 | 1.262 | 31 |
| 13 | 0.32±0.13 | 49.2 | 5.9 | 42 | 1.861 | 0.628 | 42 | 64.0 | 6.8 | 28 | 4.141 | 1.506 | 28 |
| 25 | 0.65±0.16 | 44.2 | 5.4 | 38 | 1.385 | 0.471 | 38 | 54.9 | 9.0 | 27 | 2.921 | 1.275 | 28 |
| 50 | 1.22±0.21 | | | | | | | 34.3 | 6.1 | 34 | 0.796 | 0.392 | 34 |
| Bank A + B | | | | | | | | | | | | | |
| 0 | 0 | 52.4 | 5.5 | 94 | 2.170 | 0.653 | 94 | 73.5 | 9.6 | 59 | 5.558 | 2.007 | 59 |
| 6 | 0.13±0.06 | 51.0 | 5.9 | 92 | 2.019 | 0.638 | 92 | 67.7 | 8.0 | 60 | 4.854 | 1.543 | 60 |
| 13 | 0.32±0.13 | 48.7 | 5.8 | 88 | 1.811 | 0.602 | 88 | 64.8 | 7.3 | 53 | 4.309 | 1.521 | 53 |
| 25 | 0.63±0.17 | 42.8 | 5.2 | 82 | 1.272 | 0.453 | 82 | 55.5 | 8.9 | 54 | 2.911 | 1.249 | 54 |
| 50 | 1.24±0.25 | | | | | | | 35.7 | 8.0 | 61 | 0.930 | 0.722 | 61 |

*s is the standard deviation.
**n is the number measured.

# APPENDIX 2

## TABLE 48

GROWTH RATE OF FLAGFISH AS MEASURED BY TOTAL LENGTH IN WSF OF SHALE JP-8 BIOASSAY

| Tank No. | WSF of Shale JP-8 mg/ℓ | Length of Fish on Day: 24 Length* mm | 24 s† mm | 24 N× | 46 Length mm | 46 s mm | 46 N | 60 Length mm | 60 s mm | 60 N | 74 Length mm | 74 s mm | 74 N | 97 Length mm | 97 s mm | 97 N | 148 Length mm | 148 s mm | 148 N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bank A | | | | | | | | | | | | | | | | | | | |
| 1 | 0 | 4.7 | 0.4 | 20 | 8.0 | 1.5 | 20 | 10.6 | 2.2 | 20 | 14.0 | 3.8 | 20 | 17.7 | 3.8 | 19 | 34.3 | 5.0 | 30 |
| 2 | 0.08 | 4.7 | 0.5 | 20 | 7.9 | 1.2 | 20 | 11.1 | 2.2 | 20 | 14.7 | 3.2 | 20 | 20.2 | 2.6 | 28 | 35.6 | 4.7 | 30 |
| 3 | 0.19 | 4.6 | 0.5 | 20 | 7.7 | 1.5 | 20 | 10.2 | 1.5 | 20 | 13.7 | 3.1 | 15 | 19.7 | 3.6 | 23 | 35.5 | 2.5 | 32 |
| 4 | 0.51 | 4.6 | 0.5 | 20 | 7.9 | 1.3 | 20 | 11.1 | 1.9 | 20 | 12.1 | 2.8 | 20 | 20.5 | 2.7 | 21 | 33.2 | 3.9 | 29 |
| 5 | 1.33 | 4.7 | 0.4 | 20 | 7.8 | 1.0 | 10 | 10.5 | 1.6 | 13 | 13.7 | 2.2 | 14 | - | - | - | 27.5 | 4.8 | 15 |
| Bank B | | | | | | | | | | | | | | | | | | | |
| 1 | 0 | 4.7 | 0.3 | 20 | 7.3 | 1.0 | 20 | 11.4 | 1.7 | 20 | 13.2 | 3.0 | 20 | 19.8 | 3.4 | 22 | 36.6 | 6.4 | 31 |
| 2 | 0.07 | 4.6 | 0.3 | 20 | 7.5 | 1.1 | 20 | 12.0 | 2.3 | 20 | 13.0 | 3.3 | 20 | 19.3 | 3.2 | 23 | 36.4 | 4.6 | 29 |
| 3 | 0.21 | 4.6 | 0.4 | 20 | 7.8 | 1.5 | 20 | 10.5 | 2.1 | 20 | 13.3 | 3.9 | 20 | 18.3 | 3.1 | 21 | 34.3 | 3.9 | 31 |
| 4 | 0.50 | 4.4 | 0.5 | 20 | 8.3 | 1.4 | 20 | 11.1 | 1.7 | 20 | 13.2 | 2.9 | 20 | 19.6 | 2.1 | 11 | 33.3 | 4.8 | 29 |
| 5 | 1.37 | 4.6 | 0.4 | 20 | 8.0 | 1.4 | 10 | 10.9 | 1.9 | 12 | 15.6 | 2.8 | 16 | - | - | - | 29.6 | 3.5 | 14 |
| Bank A+B | | | | | | | | | | | | | | | | | | | |
| 1 | 0 | 4.7 | 0.4 | 40 | 7.7 | 1.3 | 40 | 11.0 | 2.0 | 40 | 13.6 | 3.4 | 40 | 18.8 | 3.7 | 41 | 35.4 | 5.8 | 61 |
| 2 | 0.08 | 4.6 | 0.4 | 40 | 7.7 | 1.2 | 40 | 11.6 | 2.3 | 40 | 13.9 | 3.3 | 40 | 19.8 | 2.9 | 51 | 36.0 | 4.6 | 59 |
| 3 | 0.20 | 4.6 | 0.4 | 40 | 7.7 | 1.5 | 40 | 10.3 | 1.8 | 40 | 13.4 | 3.5 | 35 | 19.0 | 3.4 | 44 | 34.9 | 3.2 | 63 |
| 4 | 0.51 | 4.5 | 0.5 | 40 | 8.1 | 1.4 | 40 | 11.1 | 1.8 | 40 | 12.6 | 2.9 | 40 | 20.1 | 2.5 | 32 | 33.2 | 4.3 | 58 |
| 5 | 1.35 | 4.6 | 0.4 | 40 | 7.9 | 1.2 | 20 | 10.7 | 1.7 | 25 | 14.7 | 2.6 | 30 | - | - | - | 28.5 | 4.3 | 29 |

* Average N measurement

† s = standard deviation

× N = number of measurements

## APPENDIX 3

### DECAY OF JP-4 IN BAY WATER (NO FISH)

An experiment was performed to determine the rate of volatility and/or biodegradation loss of the WSF of JP-4 under static bioassay conditions in the absence of fish. A 7-ℓ mixture containing 350 mℓ JP-4 and 6650 mℓ Bay water (5% JP-4) was stirred for 24 hr. After 6 hr of separation 6 ℓ of WSF were withdrawn into a 5-gal jar, covered, and gently aerated. Samples for GC analysis were taken at intervals of 0, 3, 6, 8, 9, and 24 hr.

The results (Table 49) indicate that the greatest decrease occurred between 0 and 6 hr. The initial 100% WSF was reduced by 31% after 3 hr and 62% after 6 hr. During the next 18 hr there was an additional loss of 25%.

TABLE 49

WSF OF JP-4 STATIC DECAY IN BAY WATER

| Hour | Temp., °C | JP-4 WSF mg/ℓ | % WSF Remaining |
|---|---|---|---|
| 0 | 24.0 | 15.17 | 100 |
| 3 | 23.8 | 10.41 | 69 |
| 6 | 23.5 | 5.75 | 38 |
| 8 | 20.0 | 4.30 | 28 |
| 9 | 24.0 | 3.80 | 25 |
| 24 | 16.5 | 2.03 | 13 |

These results suggest that the residence time in continuous-flow bioassay tanks should be minimized to preferably less than 3 hr in order to minimize the disparity between nominal and actual WSF concentrations.

### COMPARATIVE DECAY OF TWO WSF OF JP-4 CONCENTRATIONS (FISH PRESENT)

The WSF of JP-4 was prepared by static solubilization (1.9 ℓ JP-4 mixed with 36.1 ℓ of sea water for 20 hr and settled for 4 hr). The WSF was diluted to 75% and 65% in bioassay jars (19.5-ℓ capacity) and gentle aeration commenced. The solutions were sampled at 0 hr, and then 10 stickleback were added to each vessel to simulate bioassay conditions. The solutions were sampled after 24 hr and subjected to purge-and-trap GC analysis.

The results (Table 50) indicate the 100% WSF was 12.5 mg/ℓ and the nominal dilutions of 75% and 65% of the WSF were 5.8 and 5.5 mg/ℓ, respectively. Thus, their actual percentages were 46 and 44 or about 25% less

than nominal. Thus, the act of preparing the dilutions caused considerable volatility loss.

After 24 hr the nominal 75% solution had decreased to 2.1 mg/ℓ and the nominal 65% solution had decreased to 1.1 mg/ℓ. This indicates that there were differential decay rates and serves to explain the contrasting mortalities observed in the previous 96-hr static bioassay. With renewal of the fuel solution each 24-hr period, the difference in exposure level would be repeated.

TABLE 50

WSF OF JP-4 STATIC DECAY IN BAY WATER

| | WSF Conc., mg/ℓ | | |
|---|---|---|---|
| Hour | 100 % | 75 % | 65 % |
| 0 | 12.5 | 5.8 | 5.5 |
| 24 | | 2.1 | 1.1 |

# APPENDIX 4

## BIODEGRADATION OF SHALE JP-8

A curious sidelight of the WSF of shale JP-8 continuous-flow bioassays was the appearance of a yellow coloration in the bioassay tanks. This color would appear each week on the fourth or fifth day after the solubilizer was cleaned and replenished with fresh shale JP-8. The color intensity was a function of fuel concentration – nonexistent in the control and incrementally more intense at each successively higher fuel concentration. Whether this phenomenon could be ascribed to chemical or biological forces was investigated by studying the relation of DO to color formation. The DO of the dilution water and the solubilizer product was measured daily for 24 days.

The results are presented in Figure 9. The DO of the dilution water remained relatively constant at 8.8 ± 0.2 mg/ℓ. In contrast the DO of the solubilizer product showed a cycling pattern related to fuel replenishment. After replenishment the DO rose to 9.2 mg/ℓ and remained at that level for several days before declining to approximately 7.9 mg/ℓ by the end of the week. This decline in DO coincided with the formation of the yellow color.

These results strongly suggest that biological activity is responsible. The lag period following replenishment and the precipitous decline thereafter of DO follows a pattern consistent with a burgeoning biological growth rather than a chemical reaction.

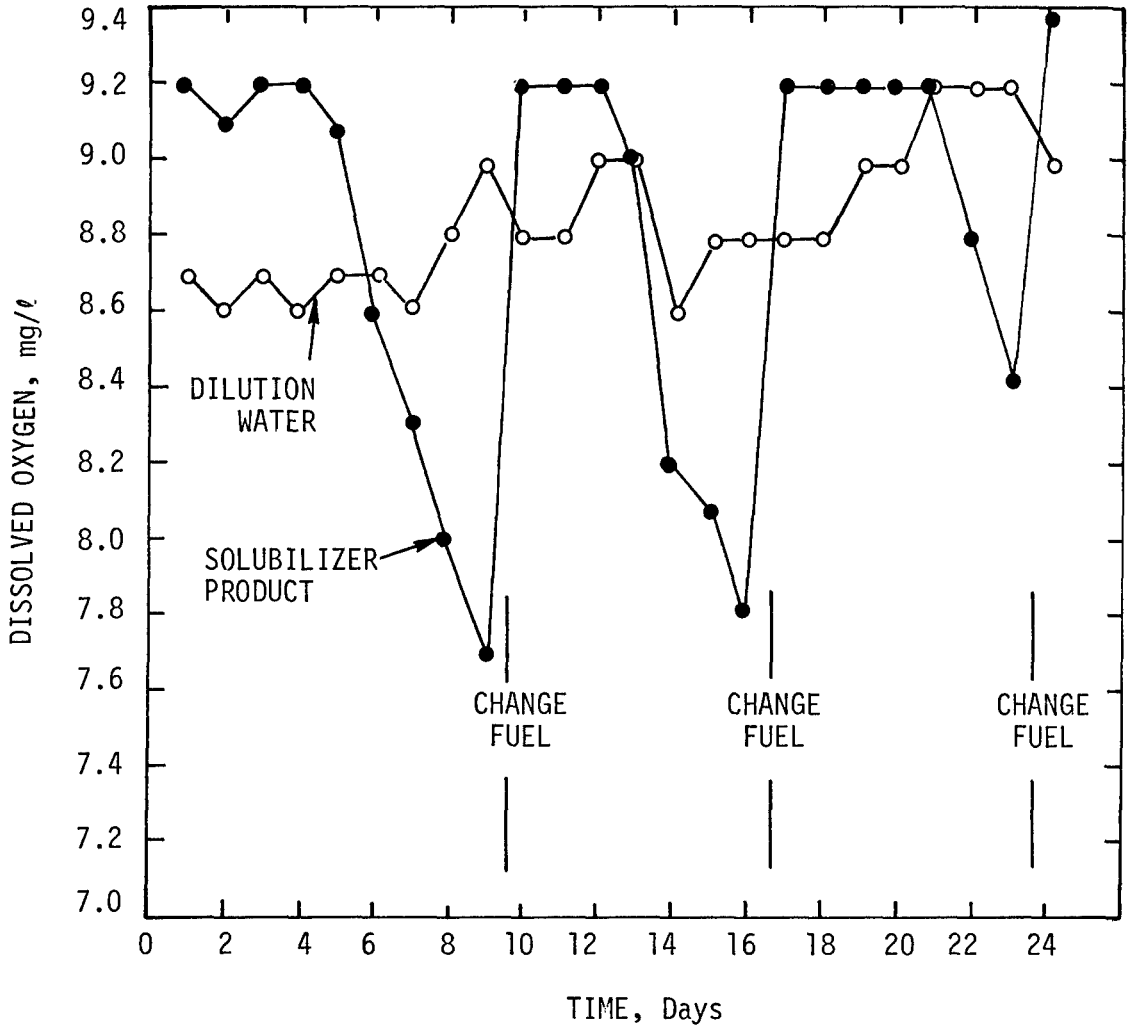



FIGURE 9. EFFECT OF SOLUBILIZER CLEANING AND REPLENISHMENT SCHEDULE ON DO OF SOLUBILIZER PRODUCT

# REFERENCES


Lerman, S., R. Cooper, J. Scherfig, and G. Greenhouse, 1975, Environmental Quality Research, Annual Report, AMRL-TR-74-82, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio. [AD A-011558]

Klein, S.A. and D. Jenkins, 1976, Environmental Quality Research, Fish and Aufwuchs Bioassay, Annual Report, AMRL-TR-76-64, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio. [AD A-033467]

Klein, S.A. and D. Jenkins, 1977, Environmental Quality Research, Fish and Aufwuchs Bioassay, Annual Report, AMRL-TR-77-54, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio [AD A-049543].

Klein, S.A. and D. Jenkins, 1978, Environmental Quality Research, Fish and Aufwuchs Bioassay, Annual Report, AMRL-TR-78-65, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio [AD A-065562].

Klein, S.A. and D. Jenkins, 1979, Environmental Quality Research, Fish and Aufwuchs Bioassay, Annual Report, AMRL-TR-79-70, Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio. [AD A-079609]

Pearson, E.A., P.N. Storrs, and R.E. Selleck, 1970, A Comprehensive Study of San Francisco Bay, Final Report, Vol. VIII Summary, Conclusions, and Recommendations, SERL Report 67-5, Sanitary Engineering Research Laboratory, University of California, Berkeley.

Krugel, S., D. Jenkins, and S.A. Klein, 1978, "Apparatus for the Continuous Dissolution of Poorly Water Soluble Compounds for Bioassays," Water Res., 12:269-272.

Anderson, J.W., J.M. Neff, B.A. Cox, H.E. Tatem, and G.M. Hightower, 1974, "Characteristics of Dispersions and Water-Soluble Extracts of Crude and Refined Oils and Their Toxicity to Estuarine Crustaceans and Fish, Marine Biol., 27:75-88.

Ehrhardt, M. and M. Blumer, 1972, "The Source Identification of Marine Hydrocarbons by Gas Chromatography," Environ. Pollut., 3:179-184.

Hedtke, S.F. and F.A. Puglisi, 1980, "Effects of Waste Oil on the Survival and Reproduction of the American Flagfish Jordanella floridae, Can. J. Fish Aquat. Sci., 37:757-764.

McKeown, B.A. and G.L. March, 1978, "The Effects of Bunker C Oil and an Oil Dispersant: Part 2 - Effects on the Accumulation of Chlorine-Labelled Bunker C Oil in Various Fish Tissues, Marine Environ. Res., 1:119-123.

Pulich, W.M., Jr., K. Winters, and C. Van Baalen, 1974, "The Effects of a No. 2 Fuel Oil and Two Crude Oils on the Growth and Photosynthesis of Microalgae, Marine Biol., 28:87-94.